```
ATGAAGGTCTCCGTGGCTGCCCTCTCCTGCCTCATGCTTGTTACTGCCCTTGGATCCCAG      60
M  K  V  S  V  A  A  L  S  C  L  M  L  V  T  A  L  G  S  Q

GCCCGGGTCACAAAAGATGCAGAGACAGAGTTCATGATGTCAAAGCTTCCATTGGAAAAT     120
A  R  V  T  K  D  A  E  T  E  F  M  M  S  K  L  P  L  E  N

CCAGTACTTCTGGACAGATTCCATGCTACTAGTGCTGACTGCTGCATCTCCTACACCCCA     180
P  V  L  L  D  R  F  H  A  T  S  A  D  C  C  I  S  Y  T  P

CGAAGCATCCCGTGTTCACTCCTGGAGAGTTACTTTGAAACGAACAGCGAGTGCTCCAAG     240
R  S  I  P  C  S  L  L  E  S  Y  F  E  T  N  S  E  C  S  K

CCGGGTGTCATCTTCCTCACCAAGAAGGGGCGACGTTTCTGTGCCAACCCCAGTGATAAG     300
P  G  V  I  F  L  T  K  K  G  R  R  F  C  A  N  P  S  D  K

CAAGTTCAGGTTTGCATGAGAATGCTGAAGCTGGACACACGGATCAAGACCAGGAAGAAT     360
Q  V  Q  V  C  M  R  M  L  K  L  D  T  R  I  K  T  R  K  N

TGA      363
*
```

FIG.1

ATGAAGATCTCCGTGGCTGCAATTCCCTTCTTCCTCCTCATCACCATCGCCCTAGGGACC
M K I S V A A I P F F L L I T I A L G T

AAGACTGAATCCTCCTCACGGGGACCTTACCACCCCTCAGAGTGCTGCTTCACCTACACT
K T E S S S R G P Y H P S E C C F T Y T

ACCTACAAGATCCCGCGTCAGCGGATTATGGATTACTATGAGACCAACAGCCAGTGCTCC
T Y K I P R Q R I M D Y Y E T N S Q C S

AAGCCCGGAATTGTCTTCATCACCAAAAGGGGCCATTCCGTCTGTACCAACCCCAGTGAC
K P G I V F I T K R G H S V C T N P S D

AAGTGGGTCCAGGACTATATCAAGGACATGAAGGAGAACTGA
K W V Q D Y I K D M K E N *

FIG.2

```
1    ATGAAGGGCCTTGCAGCTGCCCTCCTTGTCCTCGTCTGCACCATGGCCCTCTGCTCCTGT   60
     M  K  G  L  A  A  A  L  L  V  L  V  C  T  M  A  L  C  S  C

61   GCACAAGTTGGTACCAACAAAGAGCTCTGCTGCCTCGTCTATACCTCCTGGCAGATTCCA  120
     A  Q  V  G  T  N  K  E  L  C  C  L  V  Y  T  S  W  Q  I  P

121  CAAAAGTTCATAGTTGACTATTCTGAAACCAGCCCCCAGTGCCCCAAGCCAGGTGTCATC  180
     Q  K  F  I  V  D  Y  S  E  T  S  P  Q  C  P  K  P  G  V  I

181  CTCCTAACCAAGAGAGGCCGGCAGATCTGTGCTGACCCCAATAAGAAGTGGGTCCAGAAA  240
     L  L  T  K  R  G  R  Q  I  C  A  D  P  N  K  K  W  V  Q  K

241  TACATCAGCGACCTGAAGCTGAATGCCTGA   270
     Y  I  S  D  L  K  L  N  A  *
```

FIG.3

```
CKβ-8     MKVSVAALSCLMLVTALGSQARVTKDAETEFMMSKLPLENPVLLDRFHAT  50
          |.||.|||..|: . ||..| ... .|:                     |
MIP-1α    MQVSTAALAVLLCTMALCNQFSASLAAD.....................T  29

CKβ-8     SADCCISYTPRSIPCSLLESYFETNSECSKPGVIFLTKKGRRFCANPSDK  100
          ...||:|||.| || .::..||||.|:||||||||||::|..||:||:.
MIP-1α    PTACCFSYTSRQIPQNFIADYFETSSQCSKPGVIFLTKRSRQVCADPSEE  79

CKβ-8     QVQVCMRMLKLDTRIKTRKN  120
            || ::. |.|..
MIP-1α    WVQKYVSDLELSA  92
```

FIG.4

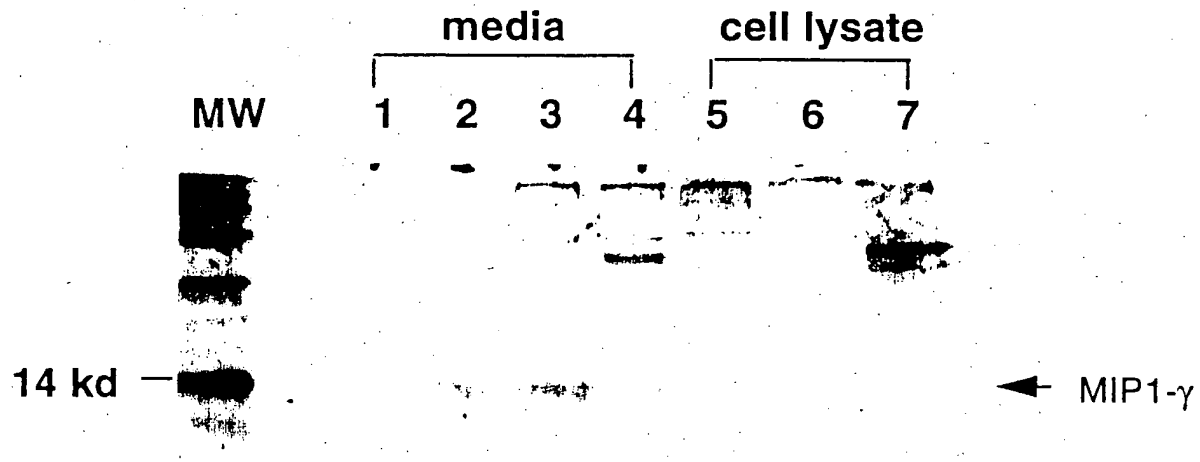
```
 1  MKGLAAALLVLVCTMALC....SCAQVGTNKELCCLVYTSWQIPQKFIVD  46
      |..  .|||  ||:||||||     |.:  .:....  ||:  |||:||||.||.|
 1  MQVSTAALAVLLCTMALCNQVLSAPLAADTPTACCFSYTSRQIPQNFIAD  50

47  YSETSPQCPKPGVILLTKRGRQICADPNKKWVQKYISDLKLNA  89
      | |||.||.||:||:||||||||:||||...|||||:|||.|.|
51  YFETSSQCSKPSVIFLTKRGRQVCADPSEEWVQKYVSDLELSA  93
```

FIG.5

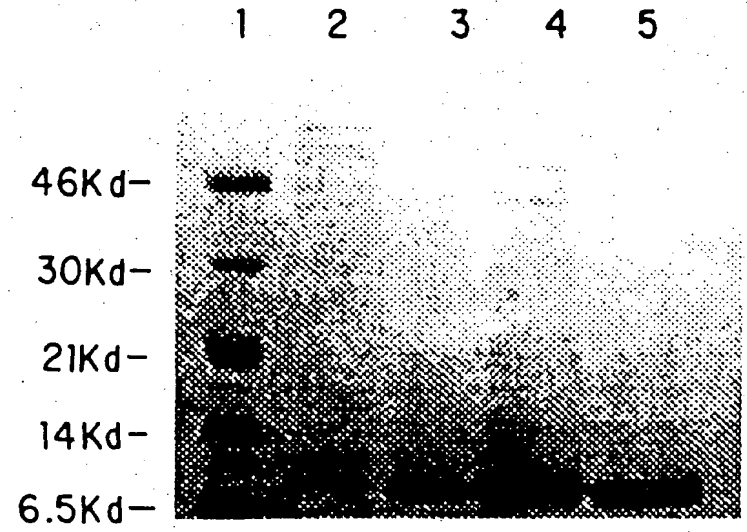
```
                .         .         .         .         .
CKβ-1       MKISVAAIPFFLLITIALGTKTESSSRGPYHPSECCFTYTTYKIPRQRIM  50
            |.:|.||:: .||.|:||... |.| :: |..|||.||. .||.. |
MIP-1α      MQVSTAALA.VLLCTMALCNQF.SASLAADTPTACCFSYTSRQIPQNFIA  48
                .         .         .         .         .
CKβ-1       DYYETNSQCSKPGIVFITKRGHSVCTNPSDKWVQDYIKDMKEN  94
            ||:||.|||||||::|:|||:: ||.:||:.|||.|:.|:.
MIP-1α      DYFETSSQCSKPGVIFLTKRSRQVCADPSEEWVQKYVSDLESA  93
```

FIG.6



1 = mock, 2 and 3 = MIP1-γ-HA, 4 = IκB-HA
5 = mock, 6 = MIP1-γ-HA, 7 = IκB-HA

FIG.7



LANE #	SAMPLE
1	LOW MW MARKERS
2	CKβ-1 BACULOVIRUS SUPERNATANT
3	HEPARIN COLUMN PURIFIED CKβ-1
4	S/M COLUMN PURIFIED CKβ-1
5	HW50 PURIFIED CKβ-1

FIG.8

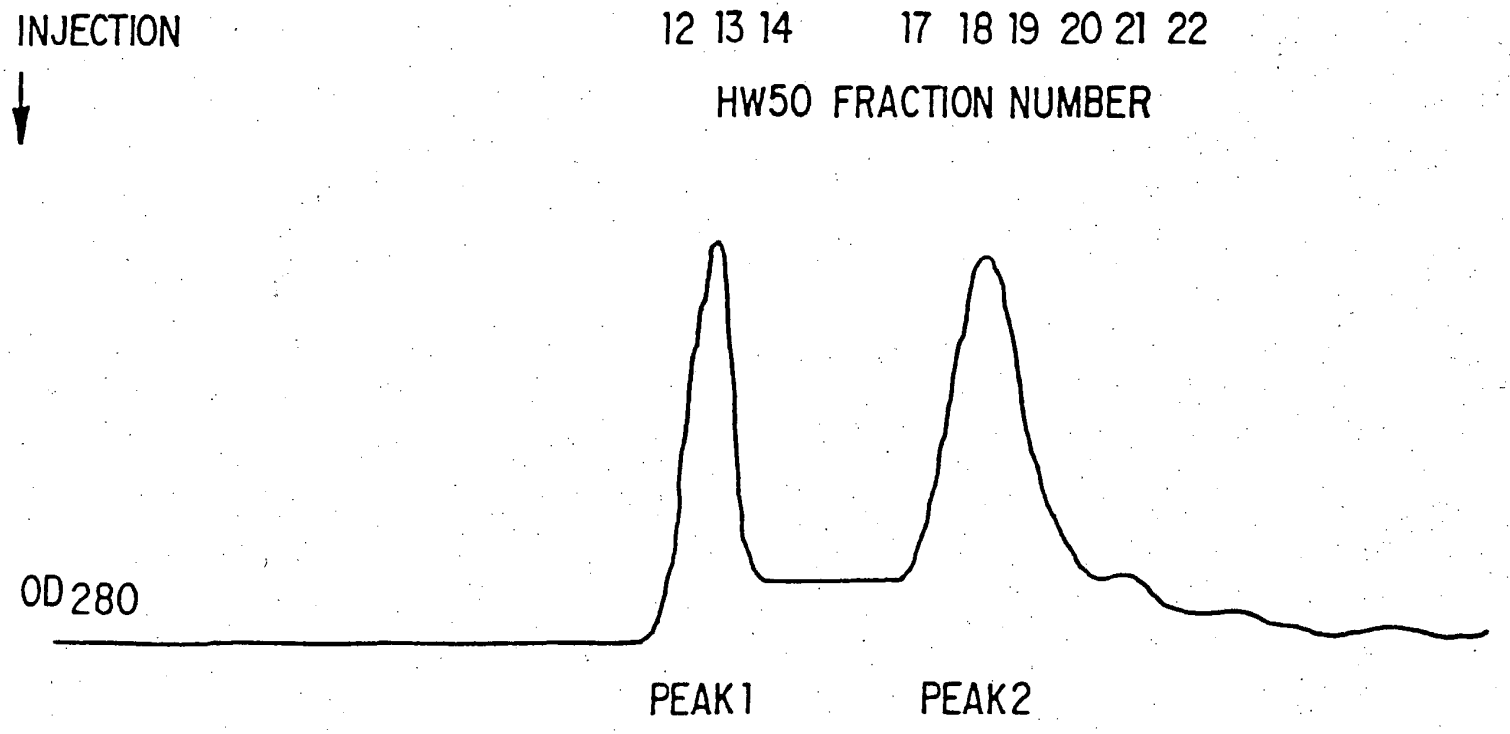
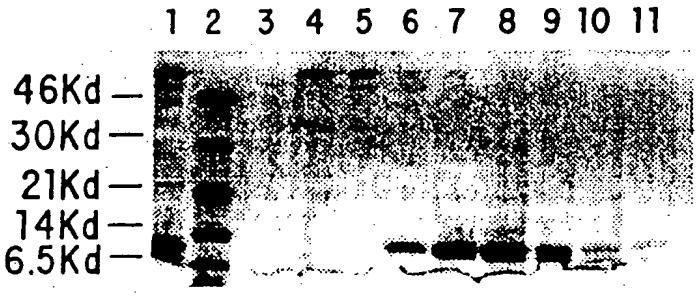

INJECTION
12 13 14
17 18 19 20 21 22
HW50 FRACTION NUMBER
OD 280
PEAK 1
PEAK 2

FIG. 9A



LANE #	SAMPLE
1	HW50 LOAD
2	LOW MW MARKERS
3	HW50 FRACTION 12
4	13
5	14
6	17
7	18
8	19
9	20
10	21
11	22

FIG.9B

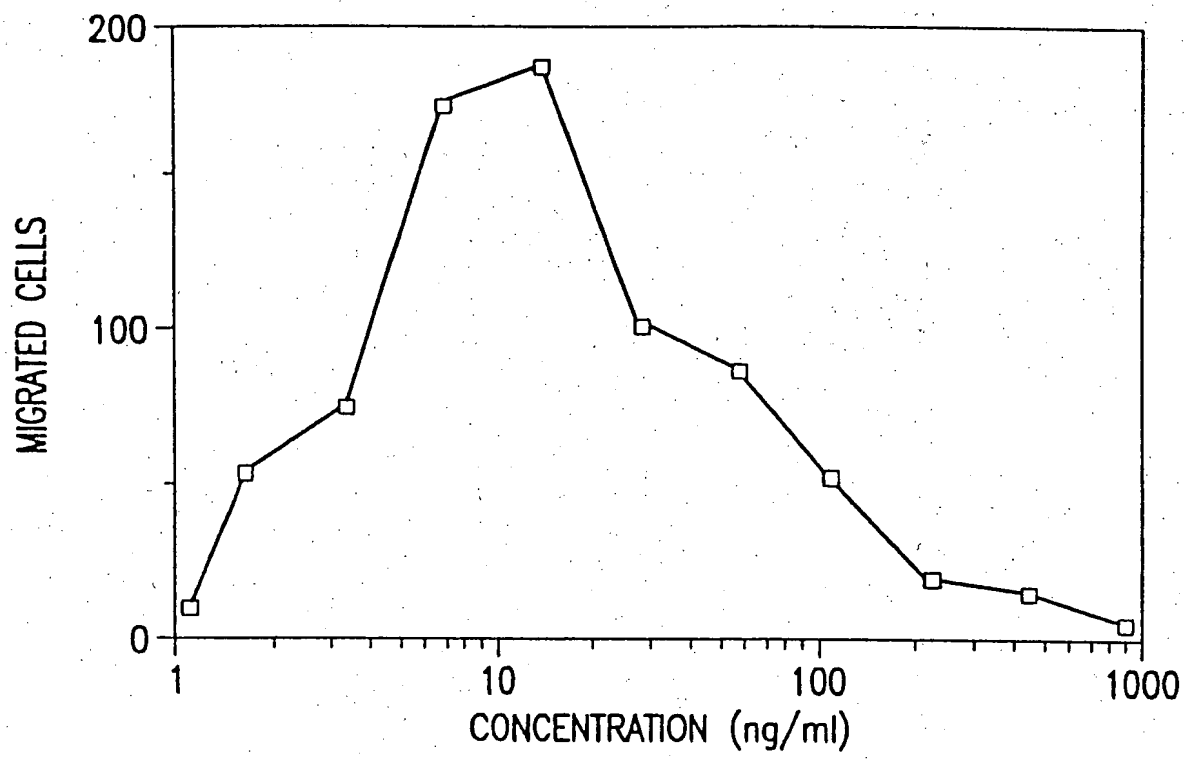


FIG.10A

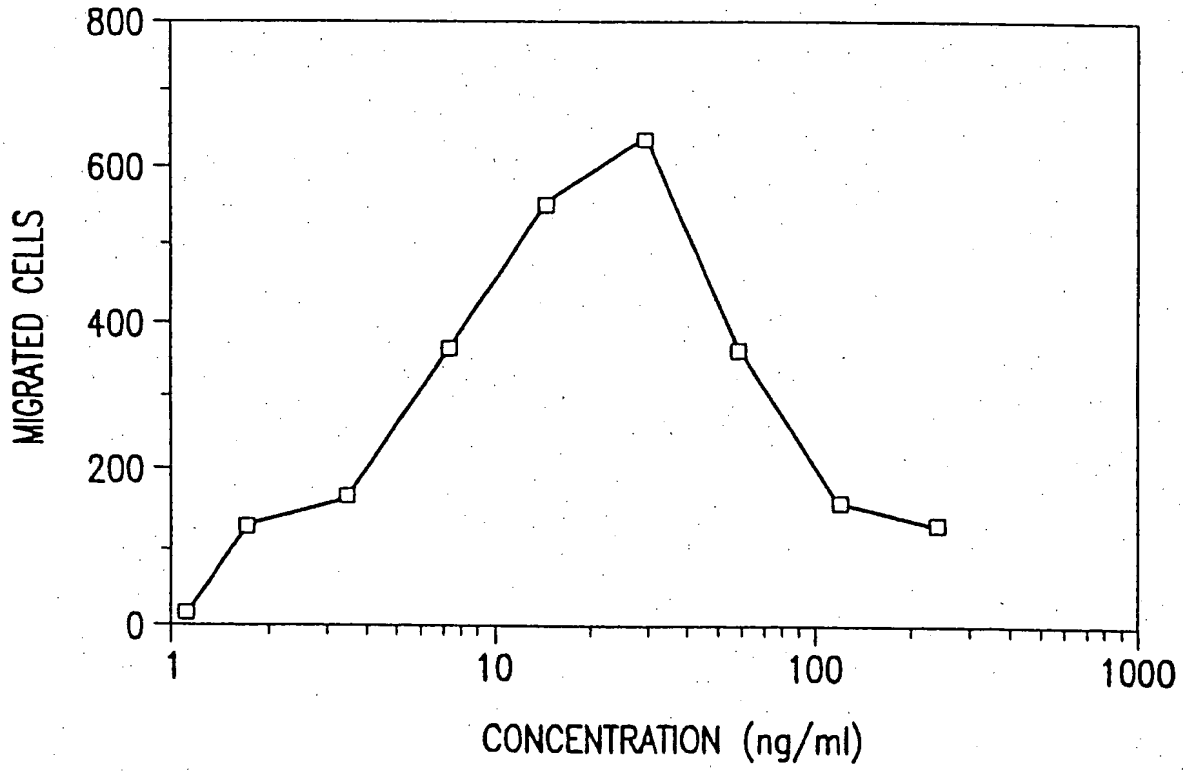


FIG.10B

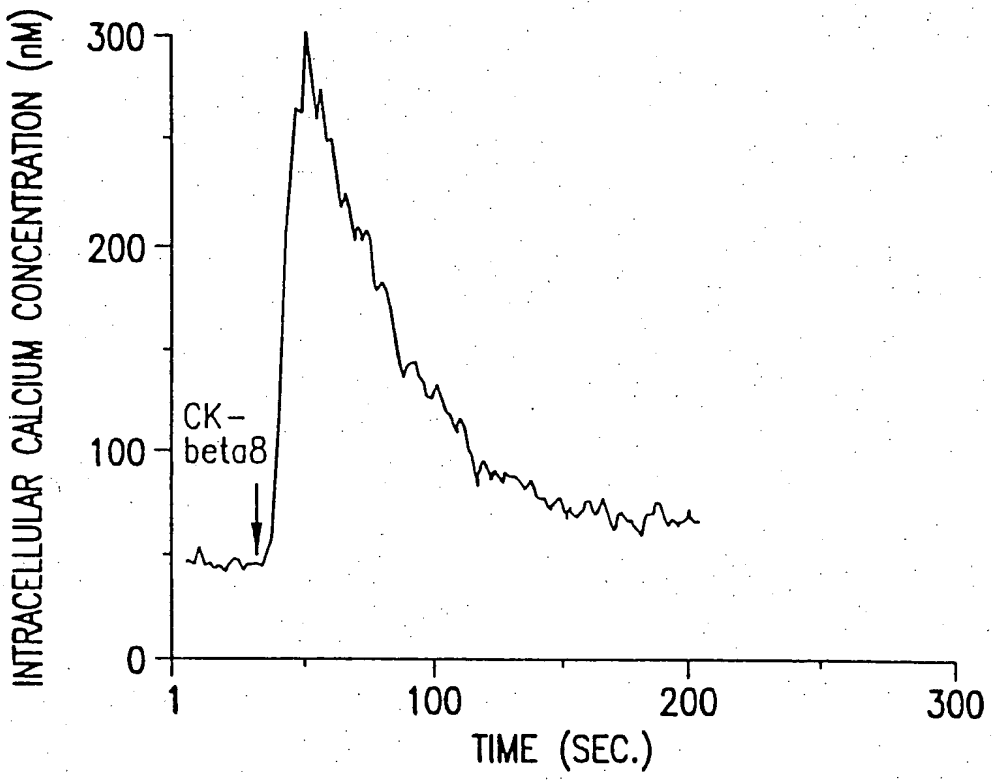

INTRACELLULAR CALCIUM CONCENTRATION (nM)
300
200
100
0
CK-
beta8
1
100
200
300
TIME (SEC.)

FIG.11

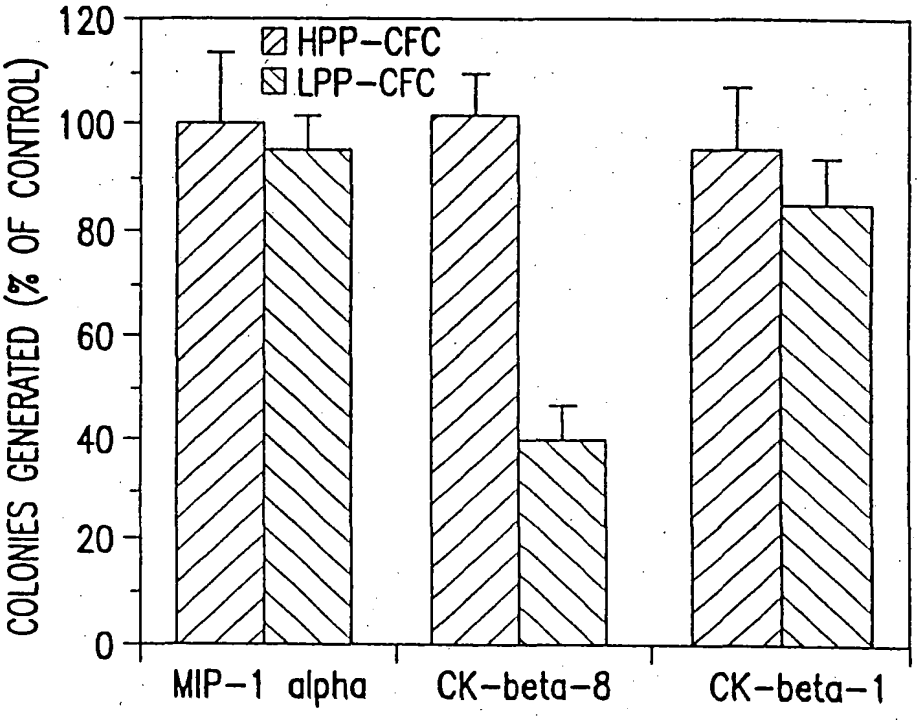

120
100
80
60
40
20
0
COLONIES GENERATED (% OF CONTROL)
HPP-CFC
LPP-CFC
MIP-1 alpha
CK-beta-8
CK-beta-1

FIG.12

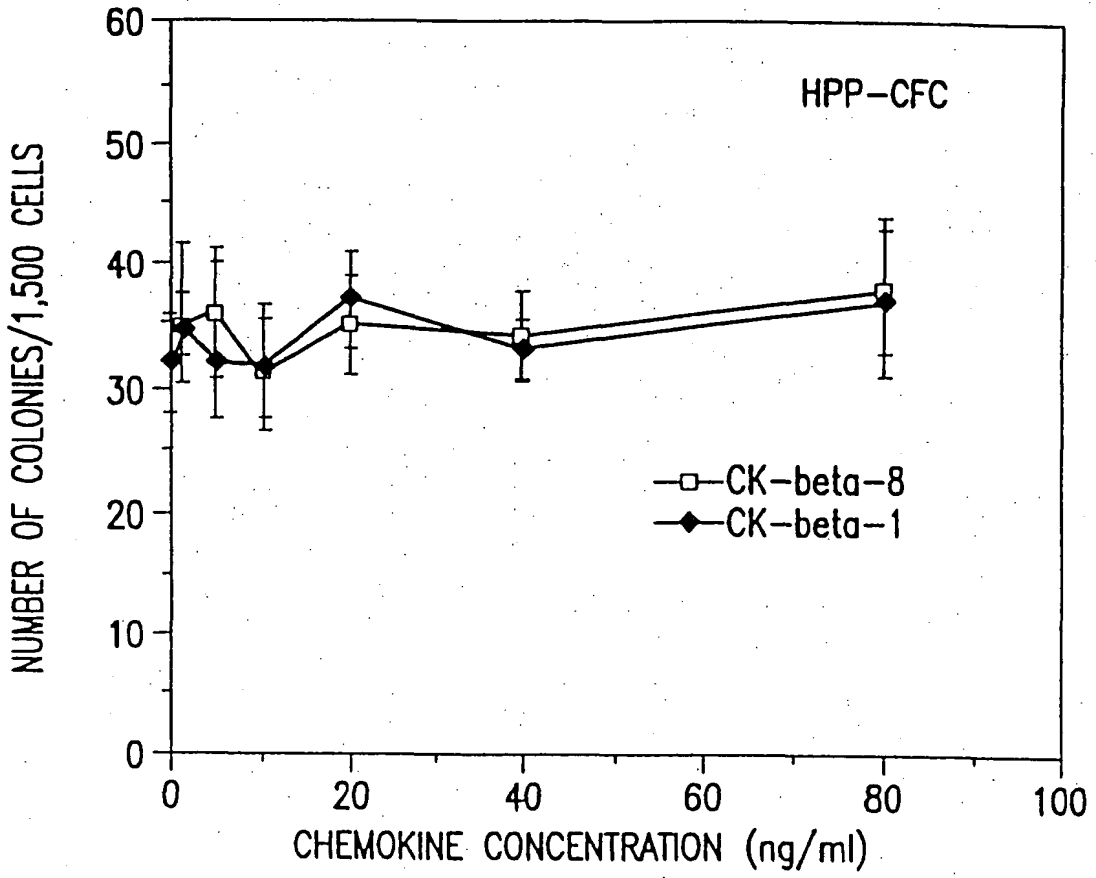


FIG.13A

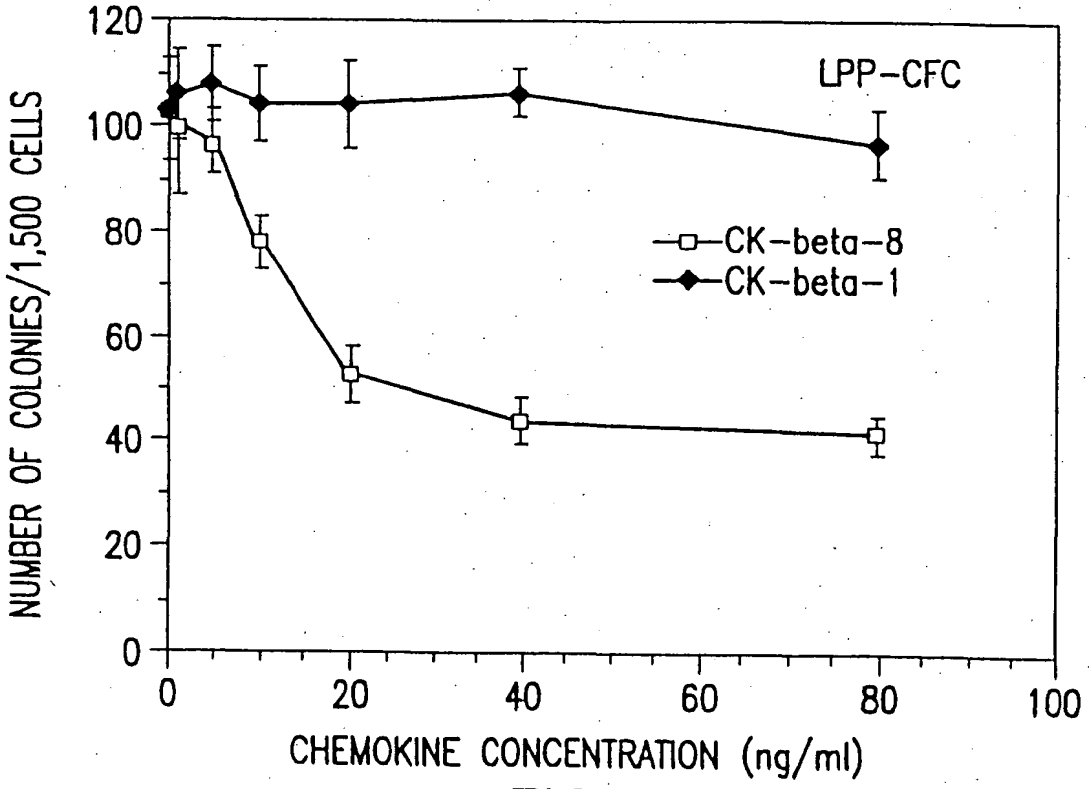


FIG.13B

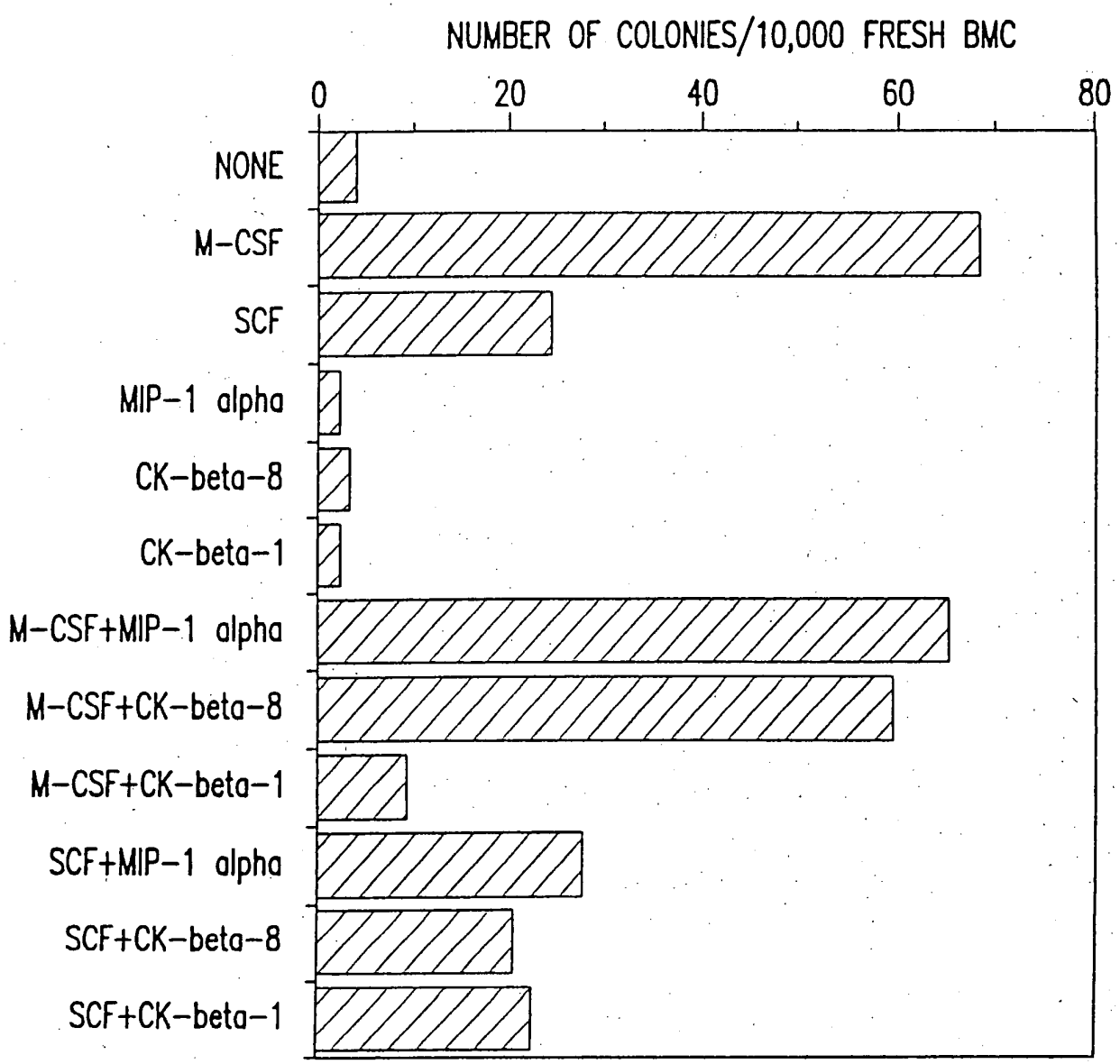

NUMBER OF COLONIES/10,000 FRESH BMC
0
20
40
60
80
NONE
M-CSF
SCF
MIP-1 alpha
CK-beta-8
CK-beta-1
M-CSF+MIP-1 alpha
M-CSF+CK-beta-8
M-CSF+CK-beta-1
SCF+MIP-1 alpha
SCF+CK-beta-8
SCF+CK-beta-1

FIG.14

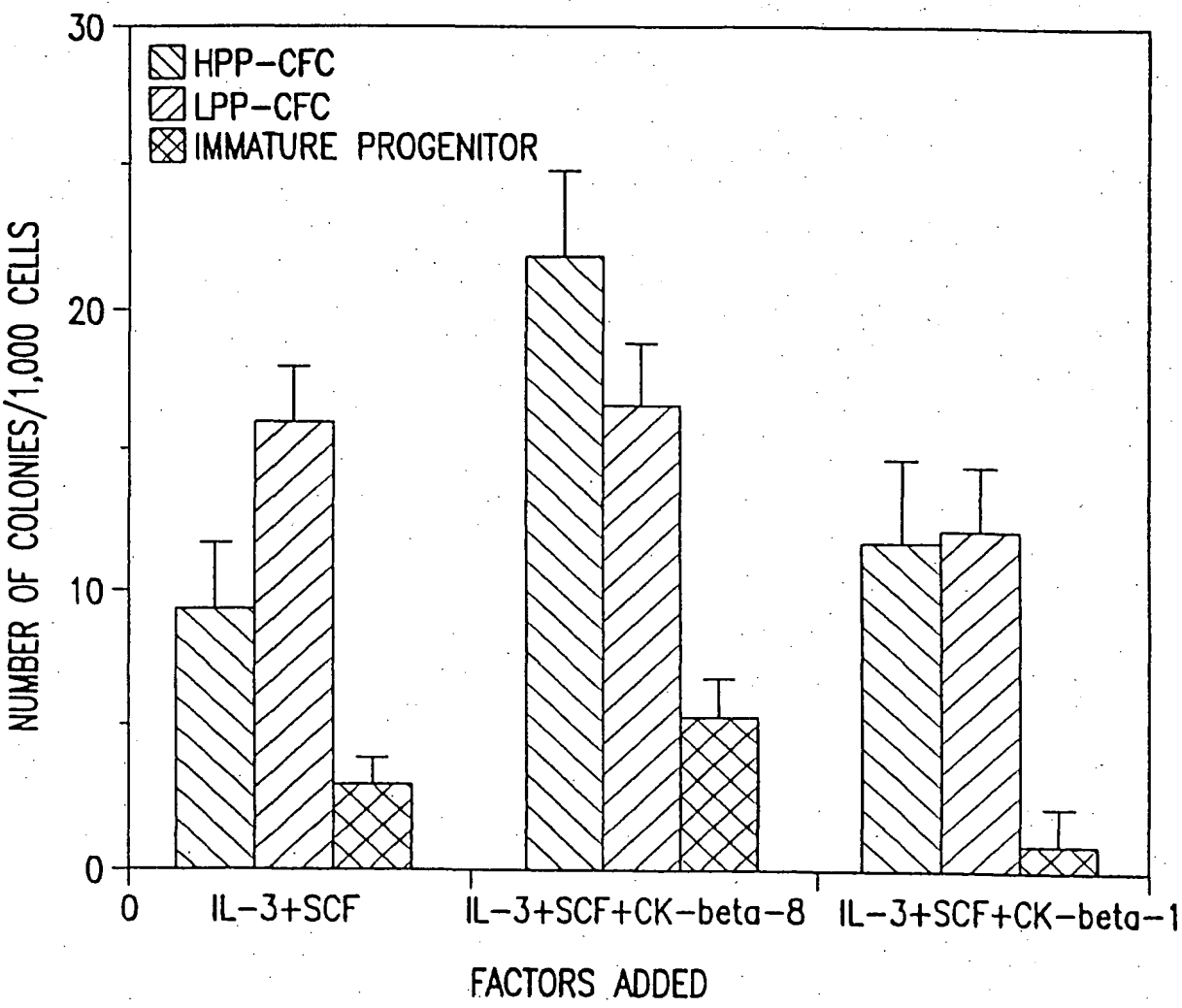

30
20
10
0
HPP-CFC
LPP-CFC
IMMATURE PROGENITOR
NUMBER OF COLONIES/1,000 CELLS
0
IL-3+SCF
IL-3+SCF+CK-beta-8
IL-3+SCF+CK-beta-1
FACTORS ADDED

FIG.15

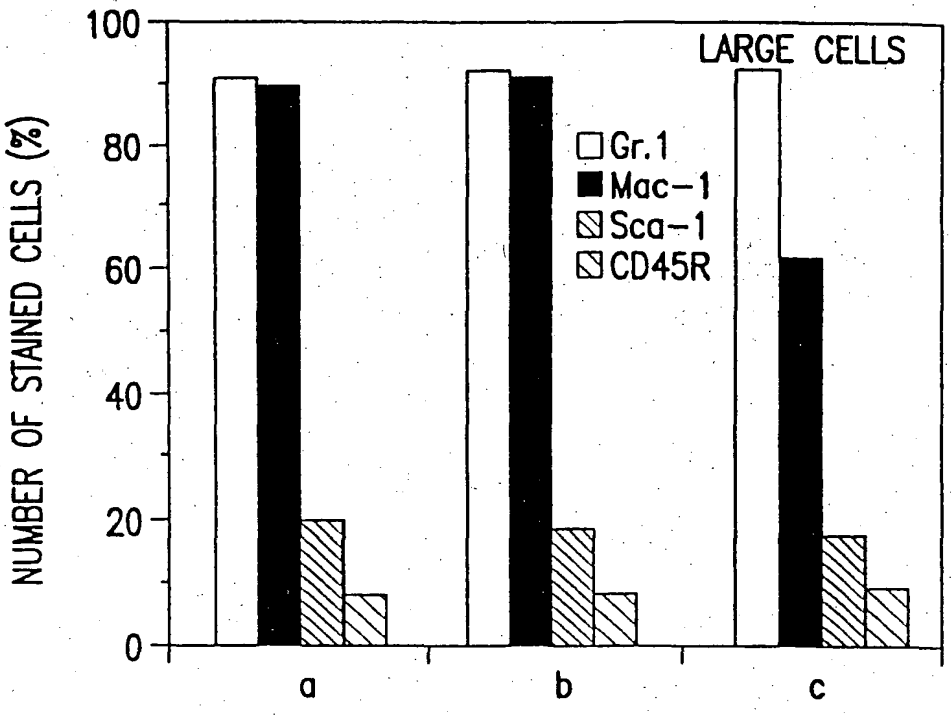


FIG.16A

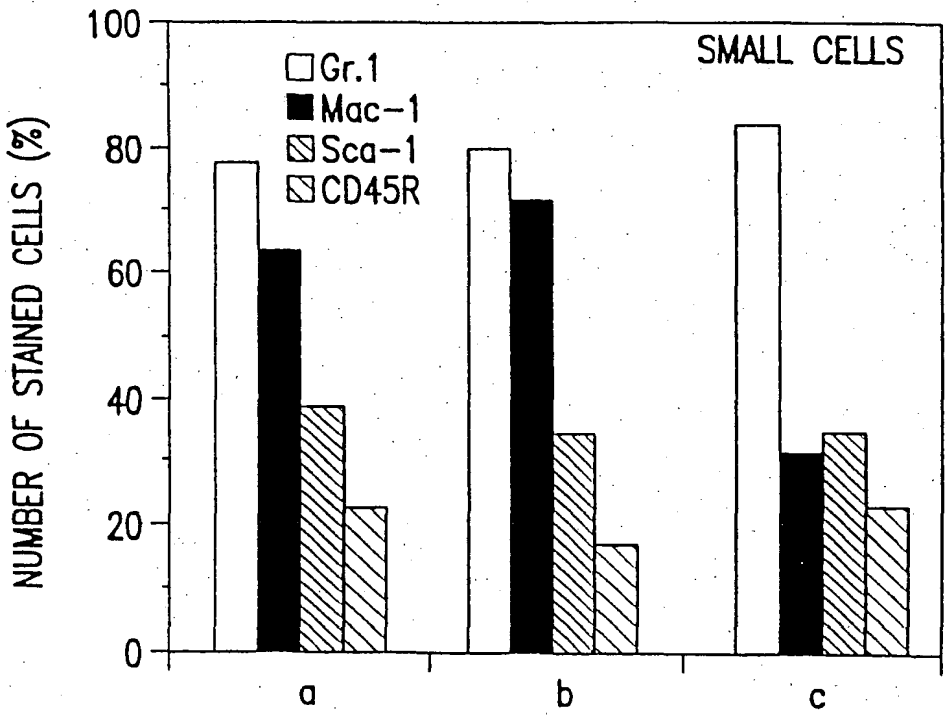


FIG.16B

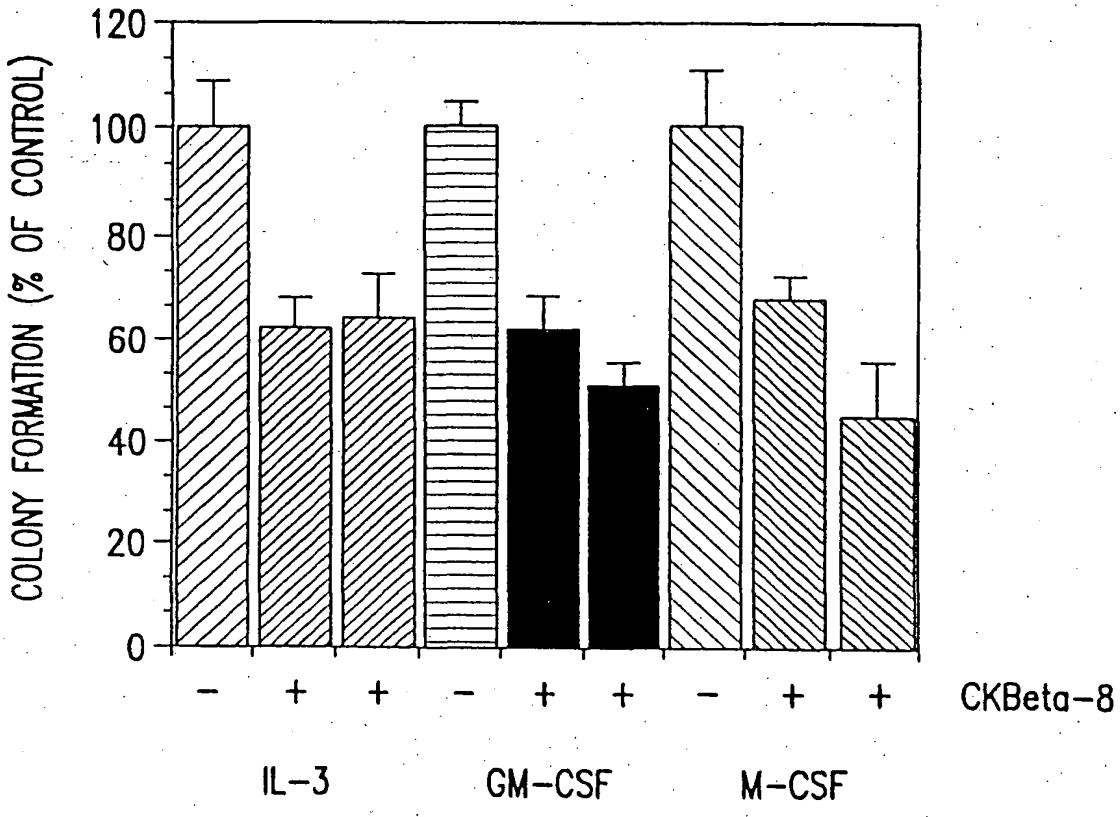

COLONY FORMATION (% OF CONTROL)
120
100
80
60
40
20
0
- + + - + + - + +
CKBeta-8
IL-3
GM-CSF
M-CSF

FIG.17

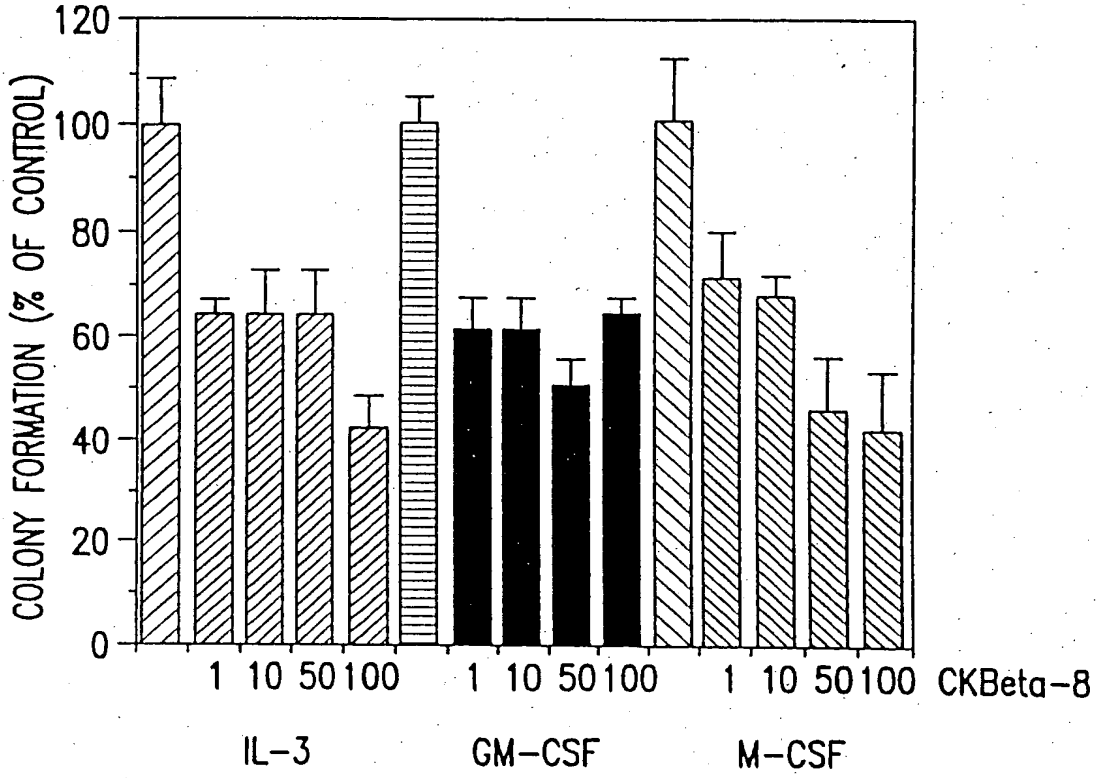

120
100
80
60
40
20
0
COLONY FORMATION (% OF CONTROL)
1 10 50 100
1 10 50 100
1 10 50 100
CKBeta-8
IL-3
GM-CSF
M-CSF

FIG.18

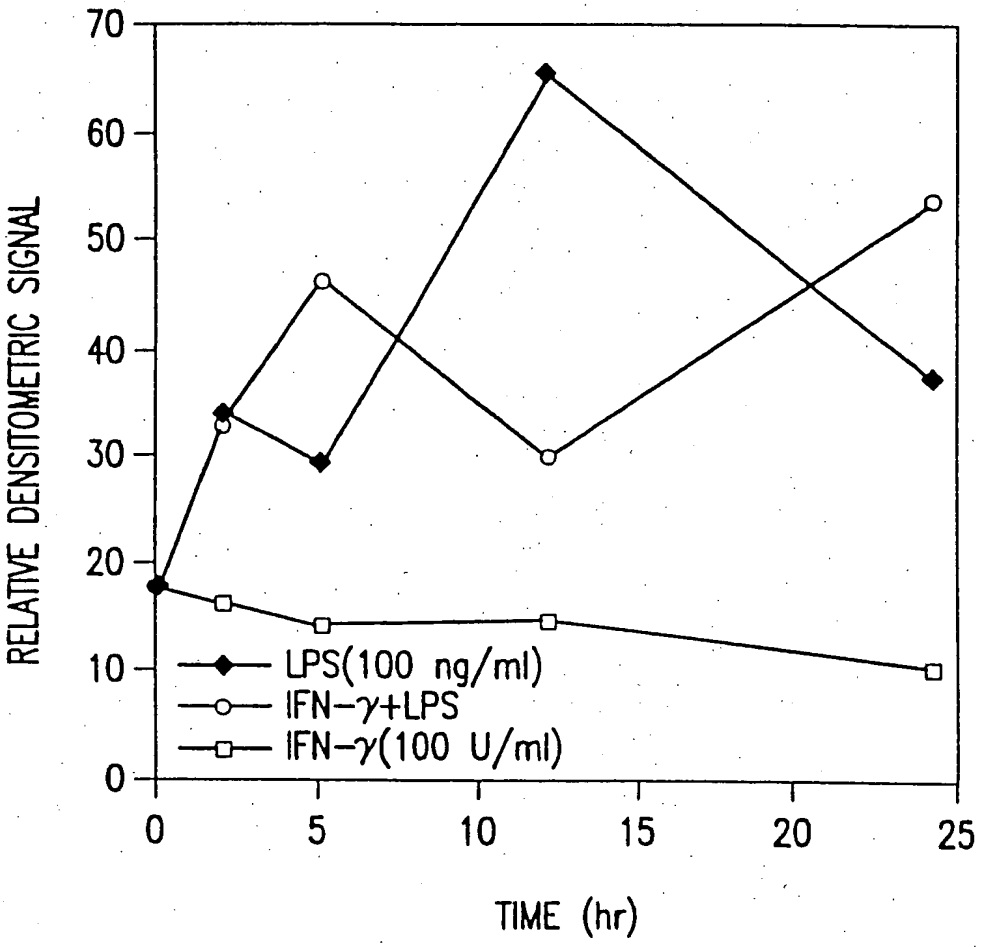
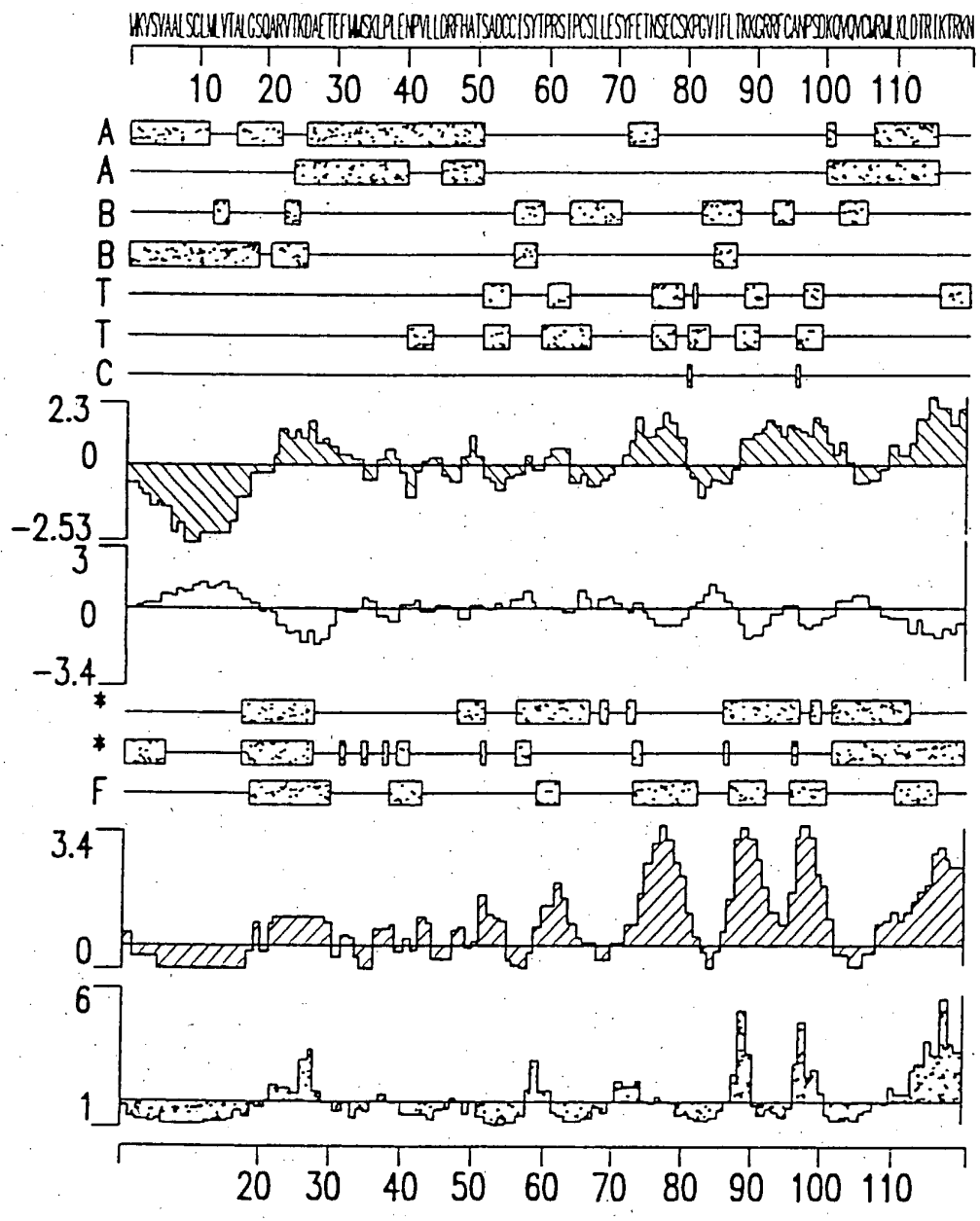

RELATIVE DENSITOMETRIC SIGNAL
70
60
50
40
30
20
10
0
0
5
10
15
20
25
TIME (hr)
LPS(100 ng/ml)
IFN-γ+LPS
IFN-γ(100 U/ml)

FIG.19



- ALPHA, REGIONS–GARNIER–ROBSON
- ALPHA, REGIONS–CHOU–FASMAN
- BETA, REGIONS–GARNIER–ROBSON
- BETA, REGIONS–CHOU–FASMAN
- TURN, REGIONS–GARNIER–ROBSON
- TURN, REGIONS–CHOU–FASMAN
- COIL, REGIONS–GARNIER–ROBSON

- HYDROPHILICITY PLOT–KYTE–DOOLITTLE

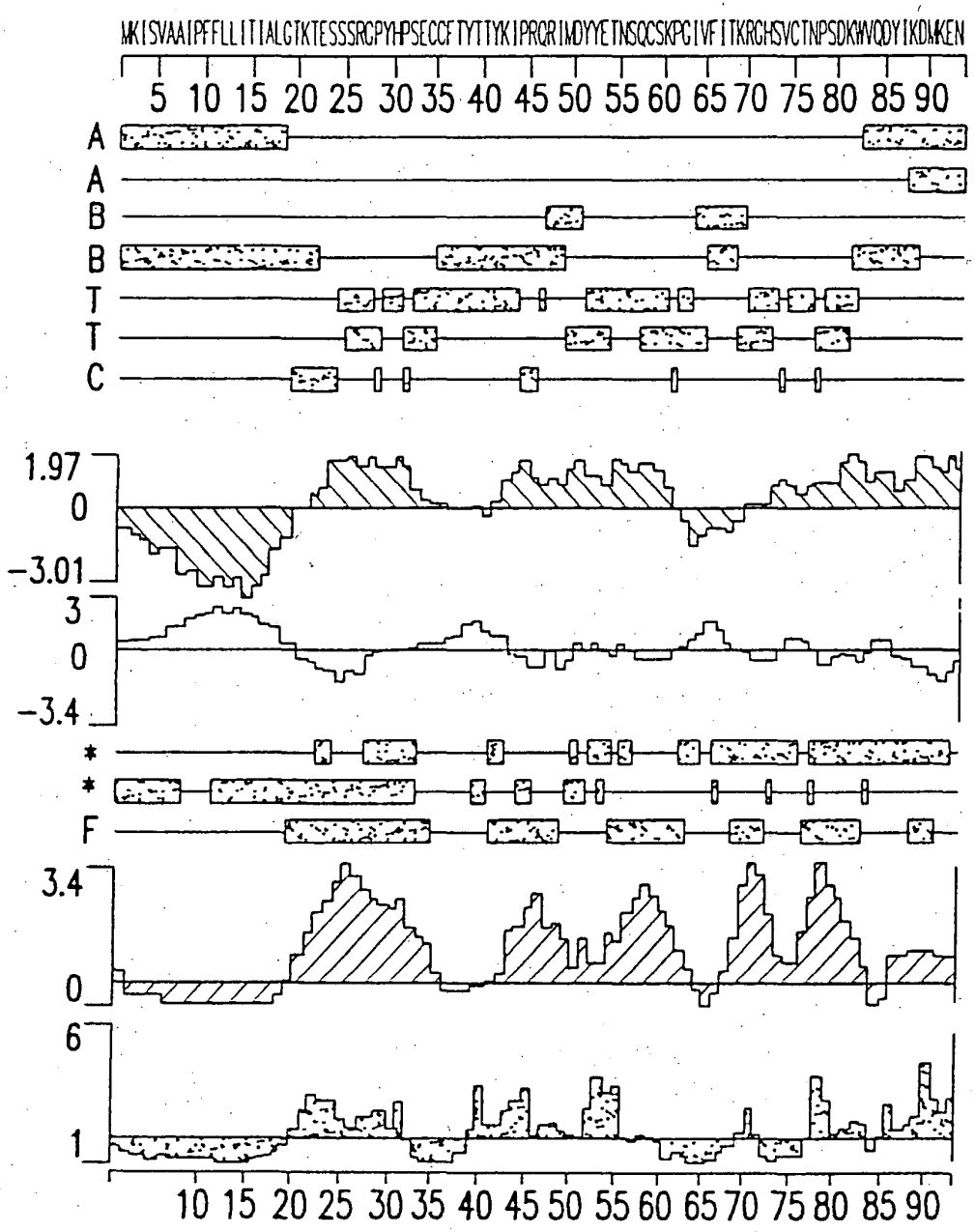
- HYDROPHOBICITY PLOT–HOPP–WOODS

- ALPHA, AMPHIPATHIC REGIONS–EISENBERG
- BETA, AMPHIPATHIC REGIONS–EISENBERG
- FLEXIBLE REGIONS–KARPLUS–SCHULZ

- ANTIGENIC INDEX–JAMESON–WOLF

- SURFACE PROBABILITY PLOT–EMINI

FIG.20A



ALPHA, REGIONS–GARNIER–ROBSON
ALPHA, REGIONS–CHOU–FASMAN
BETA, REGIONS–GARNIER–ROBSON
BETA, REGIONS–CHOU–FASMAN
TURN, REGIONS–GARNIER–ROBSON
TURN, REGIONS–CHOU–FASMAN
COIL, REGIONS–GARNIER–ROBSON

HYDROPHILICITY PLOT–KYTE–DOOLITTLE

HYDROPHOBICITY PLOT–HOPP–WOODS

ALPHA, AMPHIPATHIC REGIONS–EISENBERG
BETA, AMPHIPATHIC REGIONS–EISENBERG
FLEXIBLE REGIONS–KARPLUS–SCHULZ

ANTIGENIC INDEX–JAMESON–WOLF

SURFACE PROBABILITY PLOT–EMINI

FIG.20B

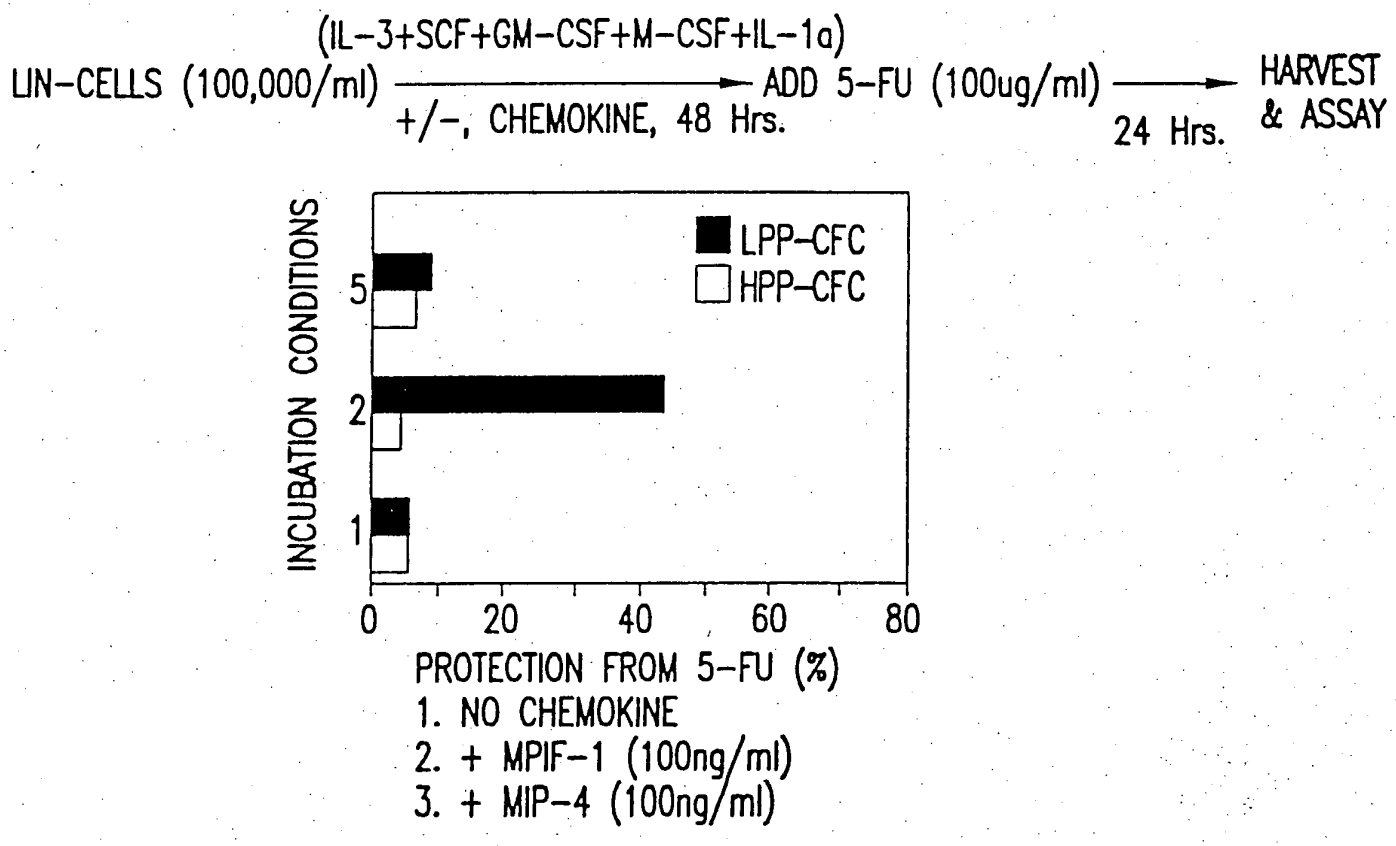

(IL-3+SCF+GM-CSF+M-CSF+IL-1a)
LIN-CELLS (100,000/ml)
+/-, CHEMOKINE, 48 Hrs.
ADD 5-FU (100ug/ml)
24 Hrs.
HARVEST & ASSAY
INCUBATION CONDITIONS
5
2
1
LPP-CFC
HPP-CFC
0
20
40
60
80
PROTECTION FROM 5-FU (%)
1. NO CHEMOKINE
2. + MPIF-1 (100ng/ml)
3. + MIP-4 (100ng/ml)

FIG.21A

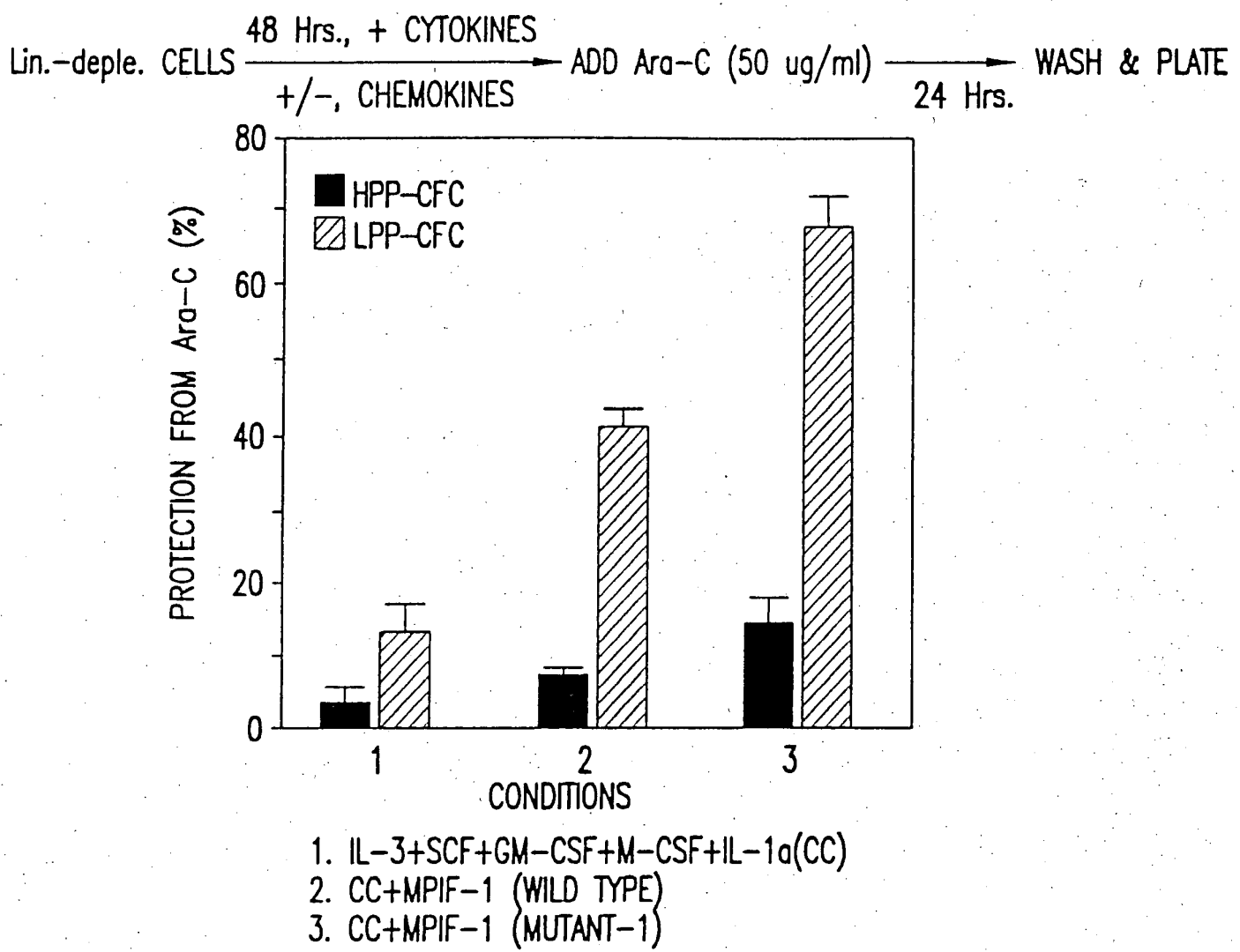

Lin.-deple. CELLS
48 Hrs., + CYTOKINES
+/-, CHEMOKINES
ADD Ara-C (50 ug/ml)
24 Hrs.
WASH & PLATE
HPP-CFC
LPP-CFC
PROTECTION FROM Ara-C (%)
0
20
40
60
80
1
2
3
CONDITIONS
1. IL-3+SCF+GM-CSF+M-CSF+IL-1a(CC)
2. CC+MPIF-1 (WILD TYPE)
3. CC+MPIF-1 (MUTANT-1)

FIG.21B

TREATMENTS	NUMBERS OF CIRCULATING WBC PER MILLILITER OF BLOOD		
	DAY 3	DAY 6	DAY 10
Gr-1 (Saline)	$8.4 \times 10^6 \pm 3.0 \times 10^6$	$10.2 \times 10^6 \pm 3.6 \times 10^6$	$7.0 \times 10^6 \pm 9.9 \times 10^5$
Gr-2, MPIF-1 ALONE	$7.8 \times 10^6 \pm 2.2 \times 10^6$ (100%)	$7.5 \times 10^6 \pm 6.5 \times 10^5$ (100%)	10.6×10^6 (100%)
Gr-3, 5-Fu ALONE	$4.23 \times 10^6 \pm 2.8 \times 10^6$ (54)	$1.8 \times 10^6 \pm 1.4 \times 10^4$ (24)	$8.8 \times 10^6 \pm 4.9 \times 10^5$ (83)
Gr-4, MPIF-1 PLUS 5-Fu	$3.49 \times 10^6 \pm 6.5 \times 10^5$ (45)	$3.98 \times 10^6 \pm 4.3 \times 10^5$ (53)	$9.48 \times 10^6 \pm 9.4 \times 10^5$ (89)

FIG.22

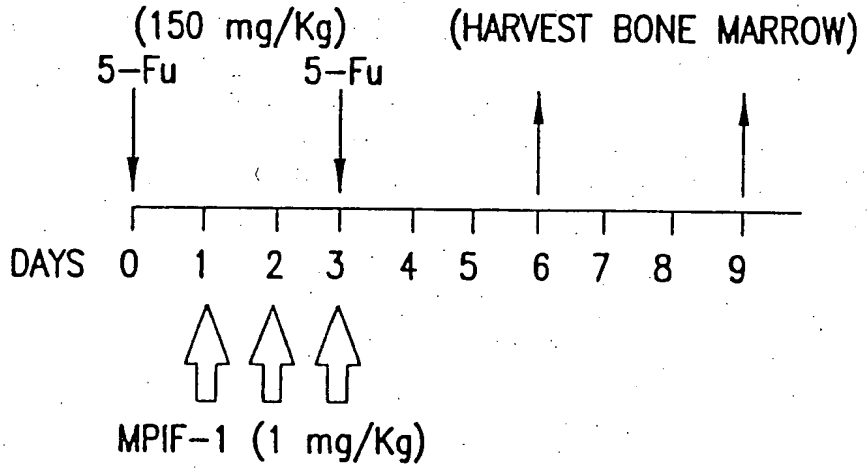

(150 mg/Kg)
5-Fu
5-Fu
(HARVEST BONE MARROW)
DAYS 0 1 2 3 4 5 6 7 8 9
MPIF-1 (1 mg/Kg)

FIG.23

GROUP	TREATMENTS	NUMBER OF COLONIES PER 2,000 CELLS DAY 6 HPP-CFC	DAY 6 LPP-CFC	DAY 9 HPP-CFC	DAY 9 LPP-CFC
1	SALINE	10.5 ± 0.7	60 ± 9.8	15 ± 2	78 ± 3.5
	SALINE	12 ± 0.7	92 ± 11	13 ± 1	80 ± 14
	SALINE	14 ± 1.4	84 ± 1.4	11 ± 2	82 ± 0
2	5-Fu	4.5 ± 3.5	3.5 ± 0.7	7 ± 2	5 ± 0
	5-Fu	12 ± 2	37 ± 16	6 ± 2	2 ± 0
	5-Fu	4 ± 2.8	6 ± 3	DEAD	DEAD
3	5-Fu PLUS MPIF-1	0	6.5 ± 3.5	16 ± 1.4	75 ± 1.4
	" " " "	0	105 ± 10	12 ± 2.8	46 ± 12
	" " " "	0	120 ± 1.4	16 ± 0	95 ± 2.8

FIG.24

```
1         10         20         30         40         50         60         70         80
MKVSVAALSC LMLVTALGSQ ARVTKDAETE FMMSKLPLEN PVLLDRFHAT SADCCISYTP RSIPCSLLES YFETNSECSK

1) Wild type:          RVTKDAE.. ......... ......... ......... ......... .........

2) Mutant-1(+1):       MRVTKDAE.. ......... ......... ......... ......... .........

3) Mutant-2(-δ 24):                              RFHAT ......... ......... .........

4) Mutant-3(-δ 23):                             DRFHAT ......... ......... .........

5) Mutant-4(-δ 26):                                HAT SAD..... ......... .........

6) Mutant-5(-δ 27):                                AT SAD..... ......... .........

7) Mutant-6(-δ 24):                             MRFHAT ......... ......... .........

8) Mutant-7(-δ 17):                       EN PVLLD..... ......... ......... .........

9) Mutant-8(-δ 22):                           LDRFHAT ......... ......... .........

10)Mutant-9(-δ 25):                          HAAGFHAT ......... ......... .........
```

FIG.25

```
gtcctcggccagccctgcctgcccaccaggaggatgaaggtctccgtggctgccctctcctgcctcatgctt
                                  M  K  V  S  V  A  A  L  S  C  L  M  L

gttactgcccttggatcccaggcccgggtcacaaaagatgcagagacagagttcatgatgtcaaagcttcca
V  T  A  L  G  S  Q  A  R  V  T  K  D  A  E  T  E  F  M  M  S  K  L  P

ttggaaaatccagtacttctggacatgctctggaggagaaagattggtcctcagatgacccttctcatgcc
L  E  N  P  V  L  L  D  M  L  W  R  R  K  I  G  P  Q  M  T  L  S  H  A

gcaggattccatgctactagtgctgactgctgcatctcctacaccccacgaagcatcccgtgttcactcctg
A  G  F  H  A  T  S  A  D  C  C  I  S  Y  T  P  R  S  I  P  C  S  L  L

gagagttactttgaaacgaacagcgagtgctccaagccgggtgtcatcttcctcaccaagaaggggcgacgt
E  S  Y  F  E  T  N  S  E  C  S  K  P  G  V  I  F  L  T  K  K  G  R  R

ttctgtgccaaccccagtgataagcaagttcaggtttgcatgagaatgctgaagctggacacacggatcaag
F  C  A  N  P  S  D  K  Q  V  Q  V  C  M  R  M  L  K  L  D  T  R  I  K

accaggaagaattgaacttgtcaaggtgaagggacacaagttgccagccaccaactttcttgcctcaactaa
T  R  K  N  *
cttcctgaattatttttttaagaagcatttattcttgtgttctggatttagagcaattcatcttttctcacc
tttaaaaaaaaaaaaaaaaaa
```

FIG.26A

```
1   MKVSVAALSCLMLVTALGSQARVTKDAETEFMMSKLPLENPVLLDMLWRR  50   MPIF-1 variant
    ||||||||||||||||||||||||||||||||||||||||||||||||||
1   MKVSVAALSCLMLVTALGSQARVTKDAETEFMMSKLPLENPVLLDR....  46   MPIF-1

51  KIGPQMTLSHAAGFHATSADCCISYTPRSIPCSLLESYFETNSECSKPGV  100
                ||||||||||||||||||||||||||||||||||||||||
47  ............FHATSADCCISYTPRSIPCSLLESYFETNSECSKPGV  83

101  IFLTKKGRRFCANPSDKQVQVCMRMLKLDTRIKTRKN  137
     ||||||||||||||||||||||||||||||||||||
 84  IFLTKKGRRFCANPSDKQVQVCMRMLKLDTRIKTRKN  120
```

FIG.26B

MPIF-1 MUTANTS	CONCENTRATION (ng/ml)
WILD TYPE	100
PREPARATION K0871	10
MUTANT-1	50
MUTANT-6	100
HG00300-B7	10
MUTANT-9	10

FIG.27

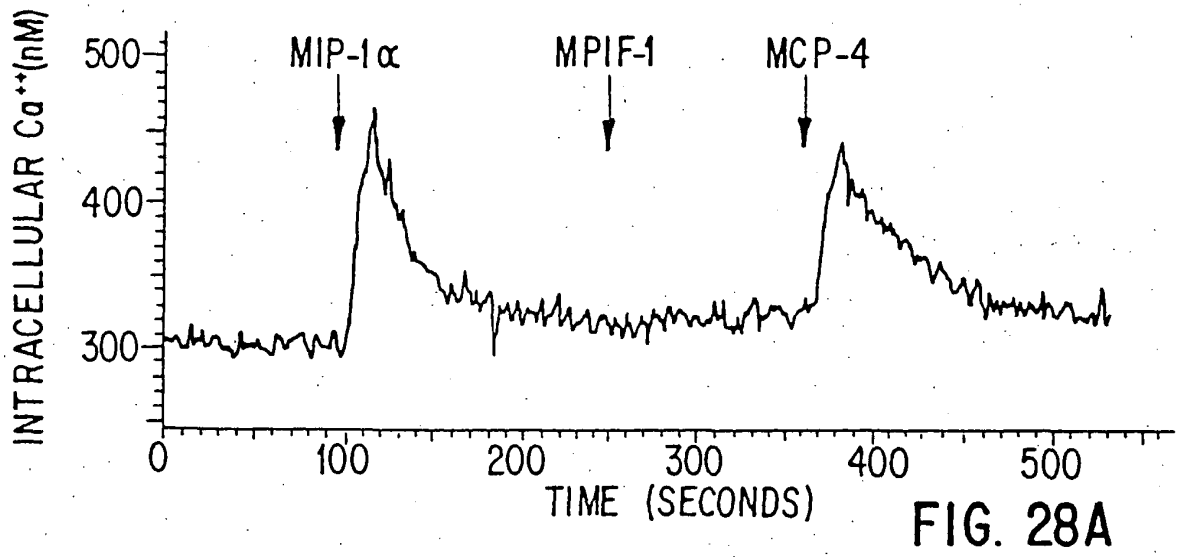


FIG. 28A

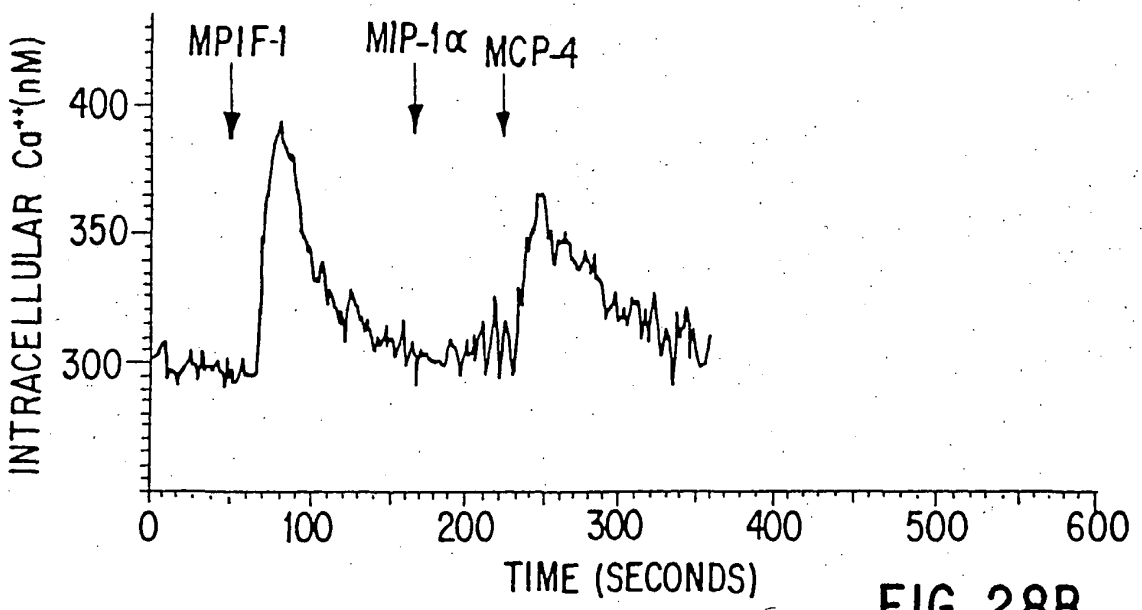


FIG. 28B

ADDITIONS	CALCIUM MOBILIZATION RESPONSE
MIP-1α ALONE	+
MPIF-1 ALONE	+
MIP-1α FOLLOWED BY MPIF-1	−
MPIF-1 FOLLOWED BY MIP-1α	−
MIP-1α FOLLOWED BY:	
PREPARATION K0871	−
HG00300-B7	−
MUTANT-6	−
MUTANT-1	−
MUTANT-9	−
PREPARATION K0871	+
K0871 FOLLOWED BY MIP-1α	−
HG00300-B7	+
HG00300-B7 FOLLOWED BY MIP-1α	−
MUTANT-6	+
MUTANT-6 FOLLOWED BY MIP-1α	−
MUTANT-1	+
MUTANT-1 FOLLOWED BY MIP-1α	−
MUTANT-9	+
MUTANT-9 FOLLOWED BY MIP-1α	−

FIG.29

PROTEINS	CHEMOTAXIS *
WILD TYPE	50–100 ng/ml (3–4X)
PREPARATION K0871	10–30 ng/ml (6–7X)
MUTANT–1	50–100 ng/ml (3–4X)
MUTANT–6	50–100 ng/ml (5–7X)
HG00300–B7	10–30 ng/ml (4–5X)

FIG.30

ADDITIONS	CONCENTRATION REQUIRED FOR 50% OF MAXIMAL LPP–CFC INHIBITION (ng/ml)
MPIF–1, WILD TYPE	10–20
MUTANT–1	15–25
MUTANT–6	1–10
PREPARATION K0871	0.1–1.0
HG00300–B7	0.1–1.0

FIG.31

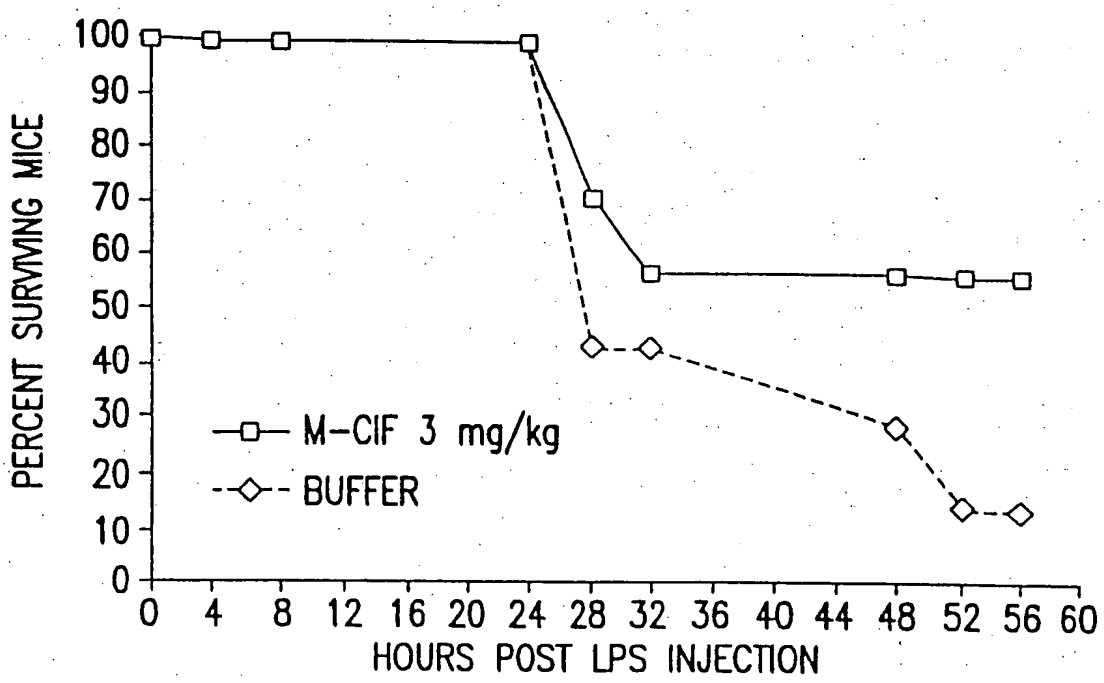

PERCENT SURVIVING MICE
100
90
80
70
60
50
40
30
20
10
0
M-CIF 3 mg/kg
BUFFER
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60
HOURS POST LPS INJECTION

FIG.32

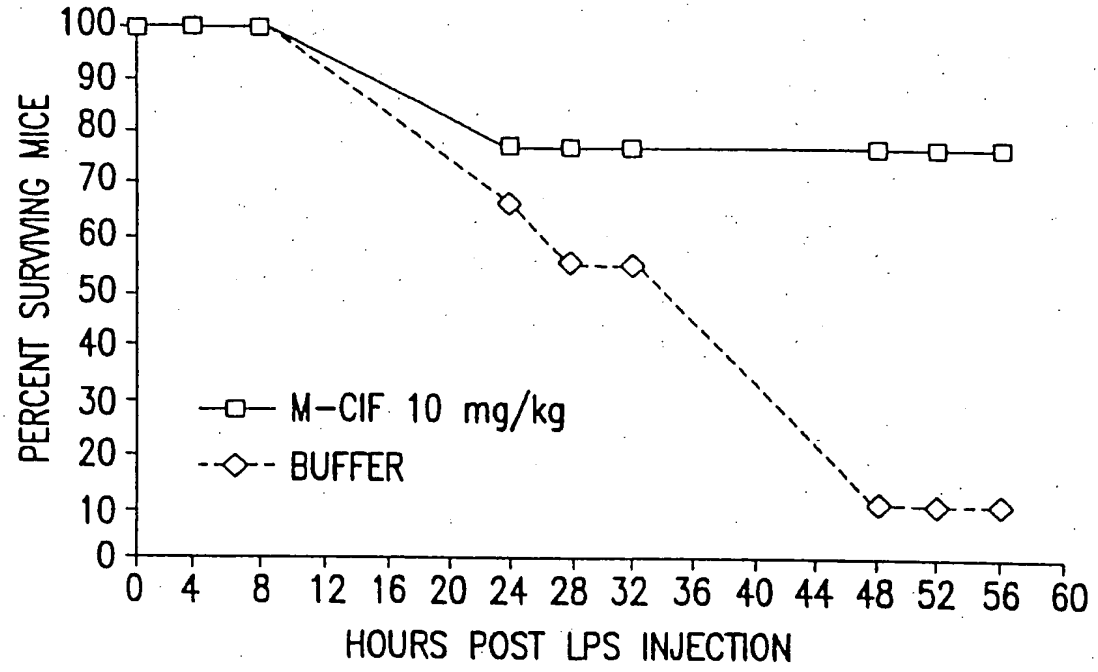

PERCENT SURVIVING MICE
100
90
80
70
60
50
40
30
20
10
0
M-CIF 10 mg/kg
BUFFER
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60
HOURS POST LPS INJECTION

FIG.33

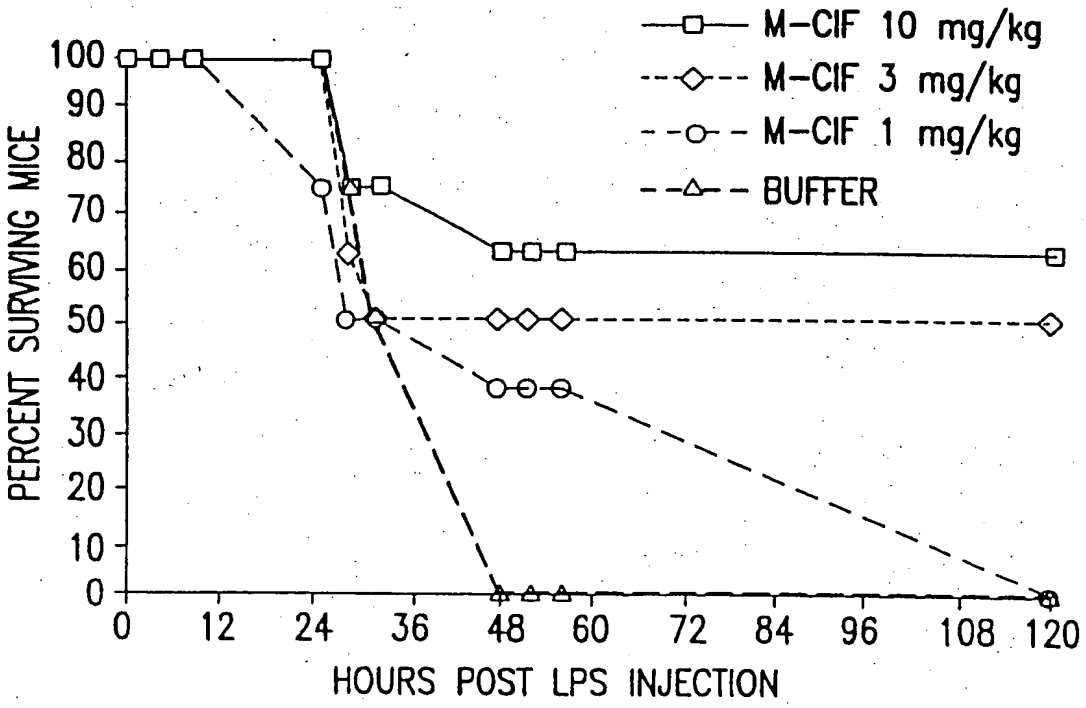


FIG.34

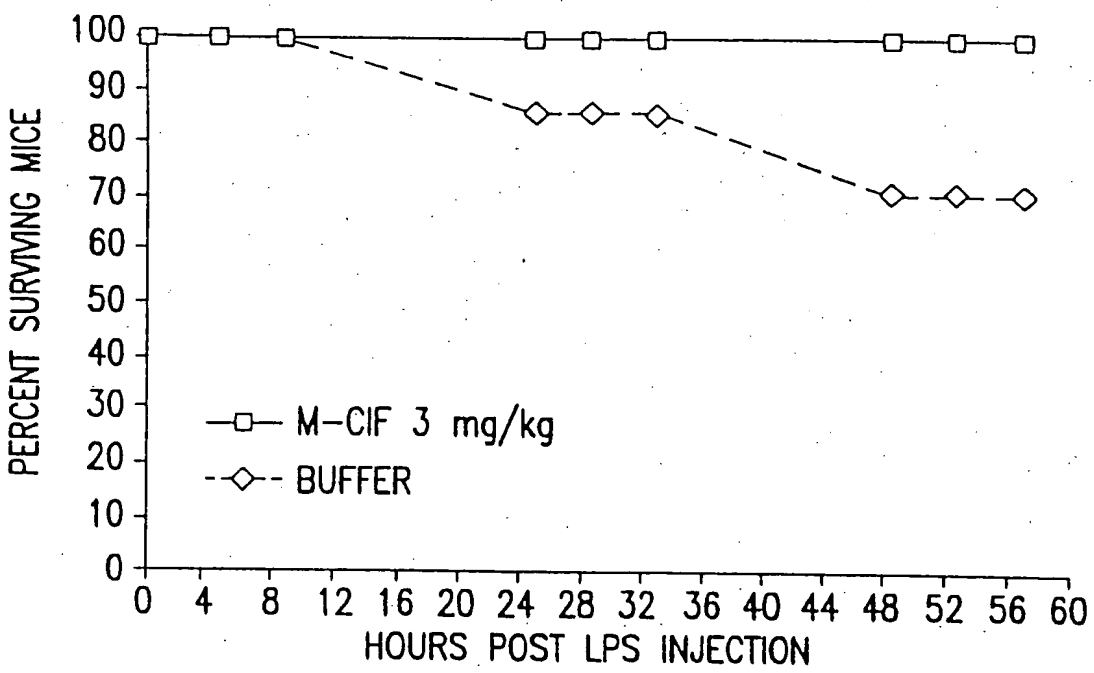


FIG.35A

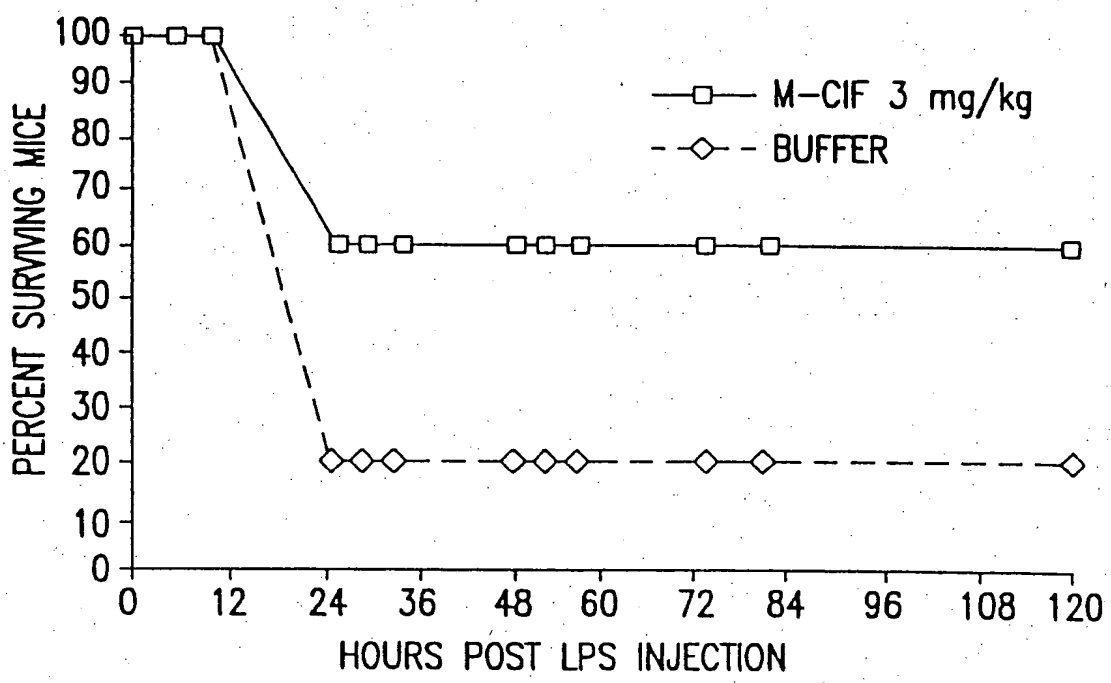


FIG.35B

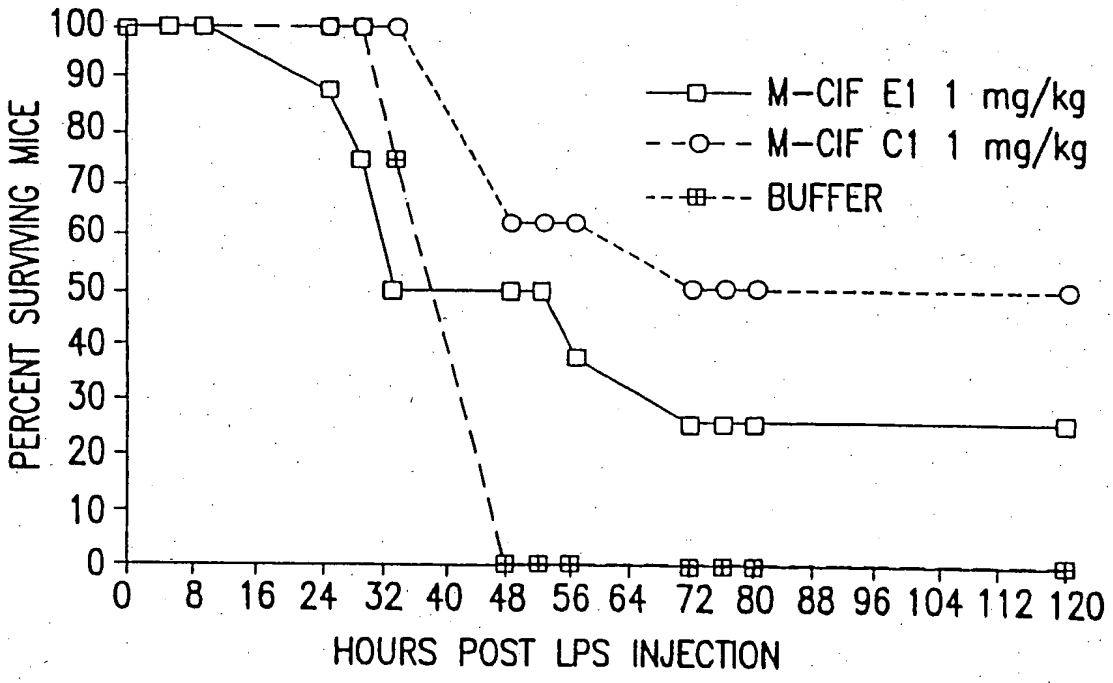


FIG.36

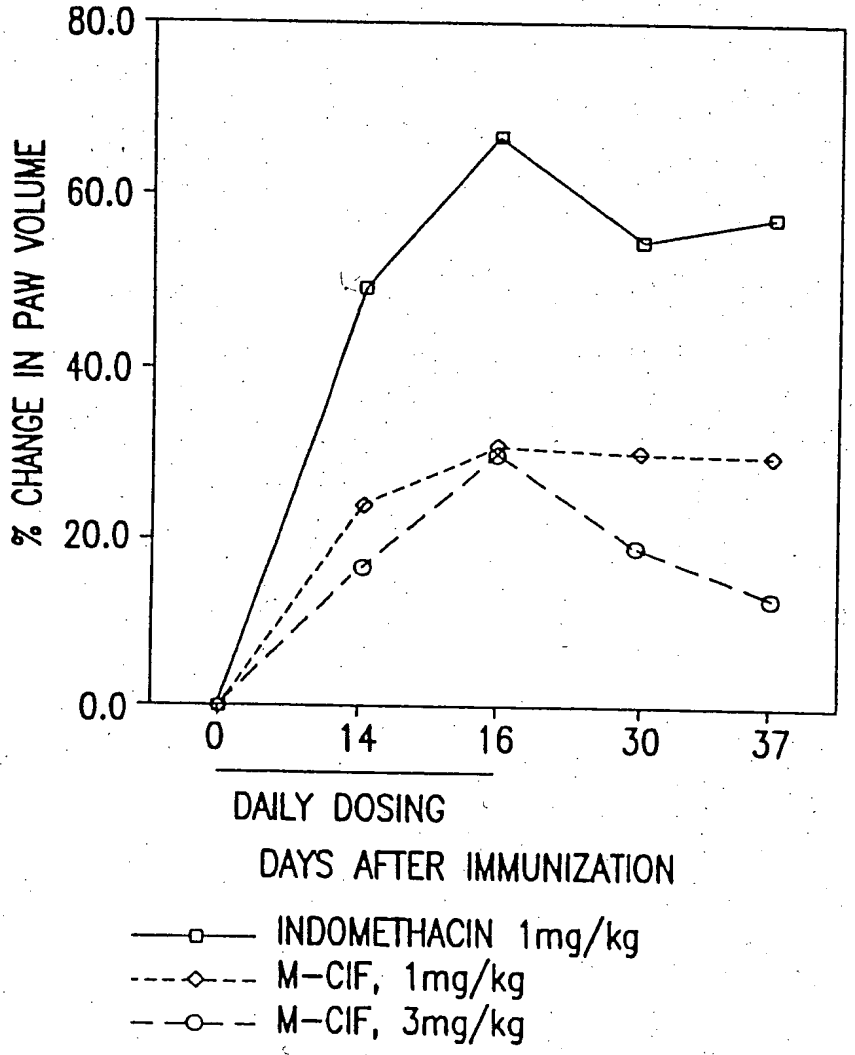

80.0
60.0
40.0
20.0
0.0
% CHANGE IN PAW VOLUME
0
14
16
30
37
DAILY DOSING
DAYS AFTER IMMUNIZATION
INDOMETHACIN 1mg/kg
M-CIF, 1mg/kg
M-CIF, 3mg/kg

FIG.37

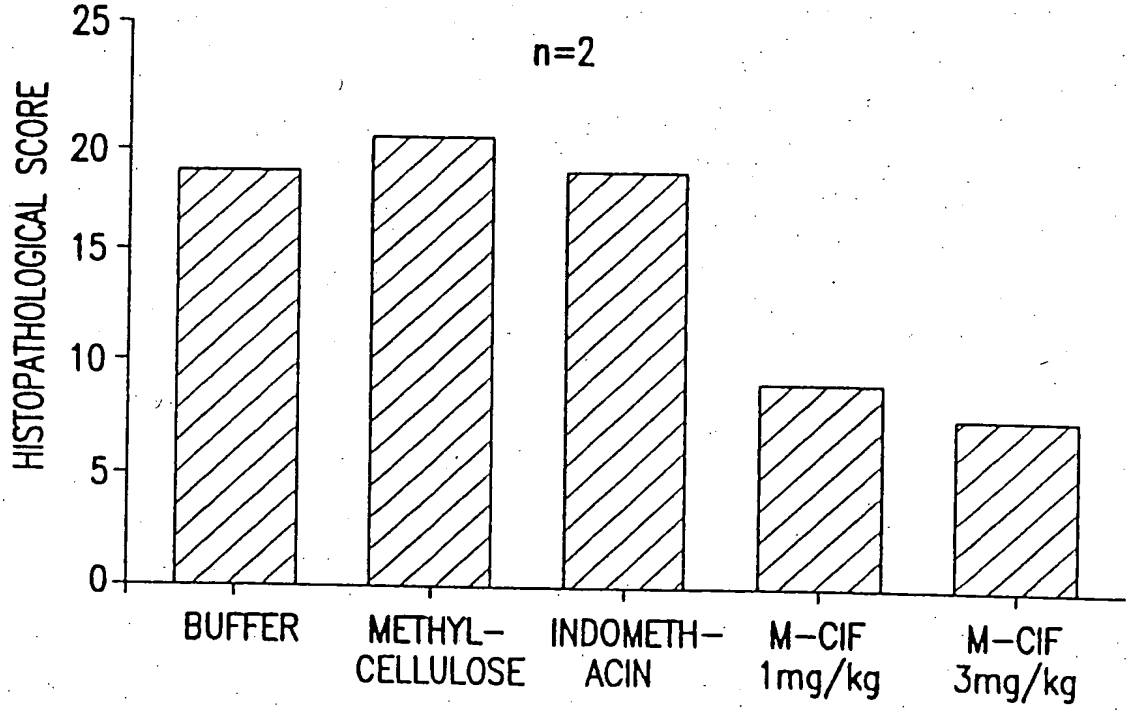

HISTOPATHOLOGICAL SCORE
25
20
15
10
5
0
n=2
BUFFER
METHYL-CELLULOSE
INDOMETH-ACIN
M-CIF 1mg/kg
M-CIF 3mg/kg

FIG.38

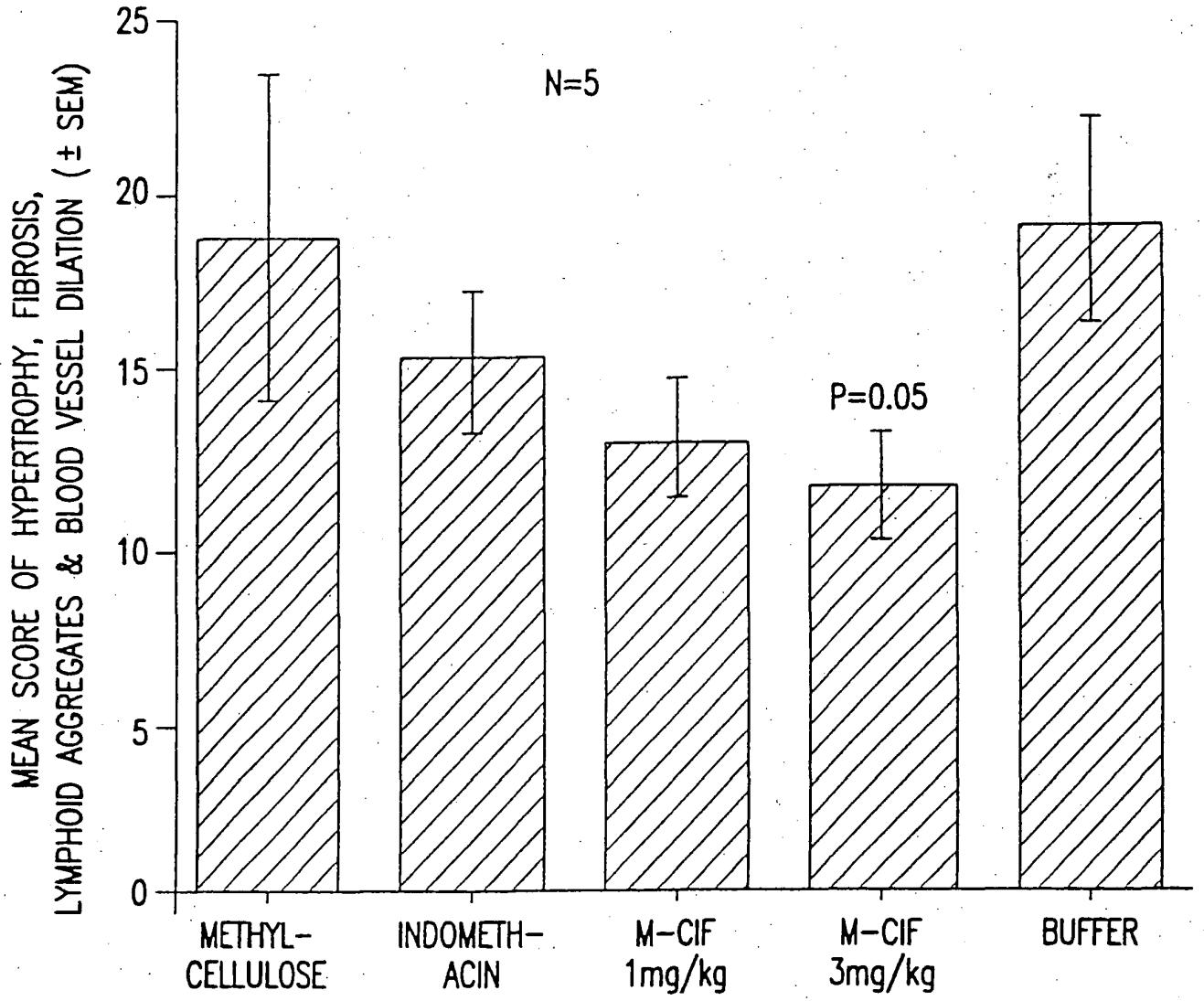

MEAN SCORE OF HYPERTROPHY, FIBROSIS, LYMPHOID AGGREGATES & BLOOD VESSEL DILATION (± SEM)
25
20
15
10
5
0
N=5
P=0.05
METHYL-CELLULOSE
INDOMETH-ACIN
M-CIF 1mg/kg
M-CIF 3mg/kg
BUFFER

FIG.39

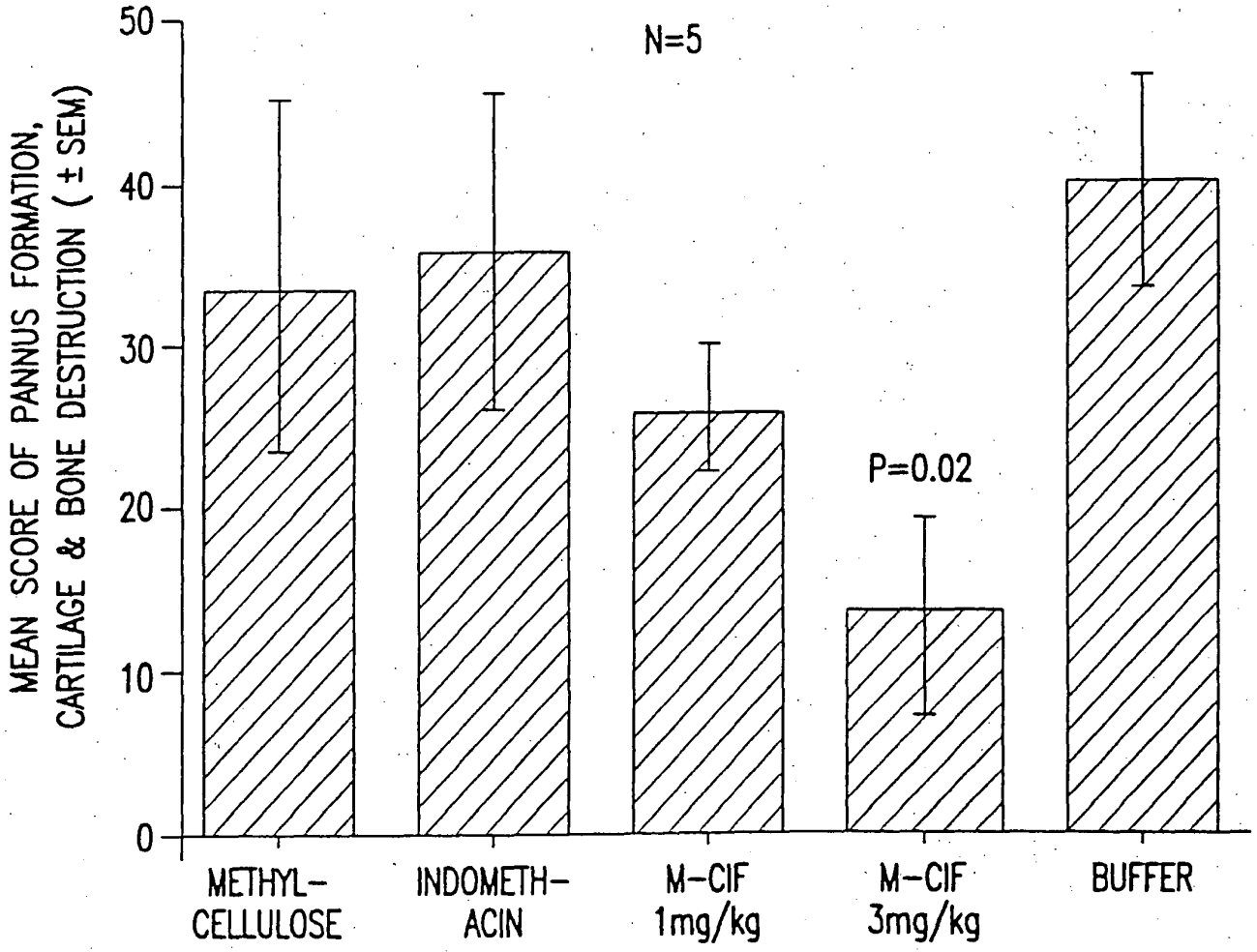

MEAN SCORE OF PANNUS FORMATION, CARTILAGE & BONE DESTRUCTION (± SEM)
50
40
30
20
10
0
N=5
P=0.02
METHYL-CELLULOSE
INDOMETH-ACIN
M-CIF 1mg/kg
M-CIF 3mg/kg
BUFFER

FIG.40

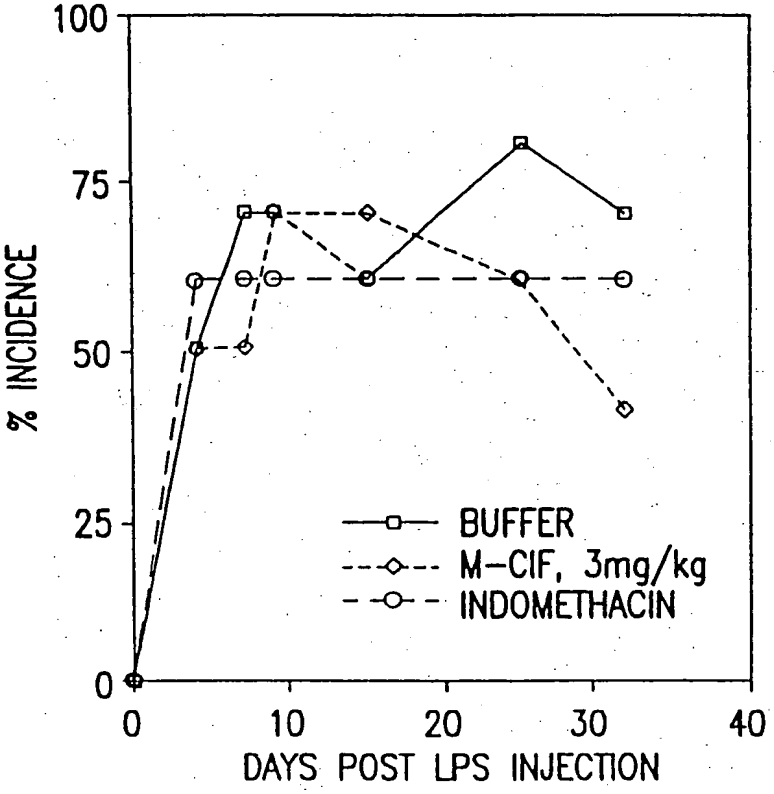

100
75
50
25
0
% INCIDENCE
0
10
20
30
40
DAYS POST LPS INJECTION
BUFFER
M-CIF, 3mg/kg
INDOMETHACIN

FIG.41

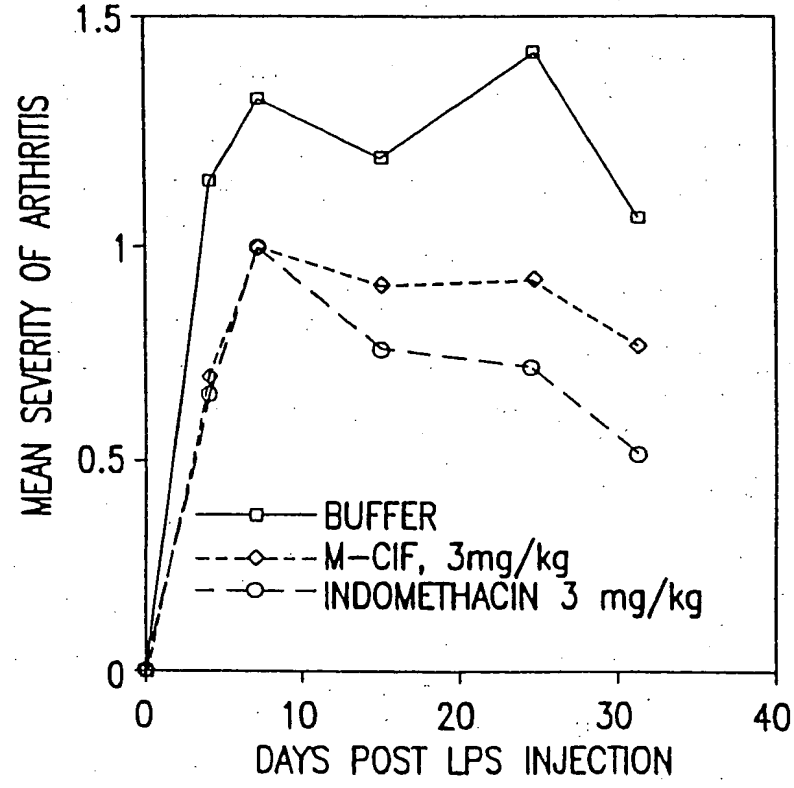

1.5
1
0.5
0
MEAN SEVERITY OF ARTHRITIS
0
10
20
30
40
DAYS POST LPS INJECTION
BUFFER
M-CIF, 3mg/kg
INDOMETHACIN 3 mg/kg

FIG.42

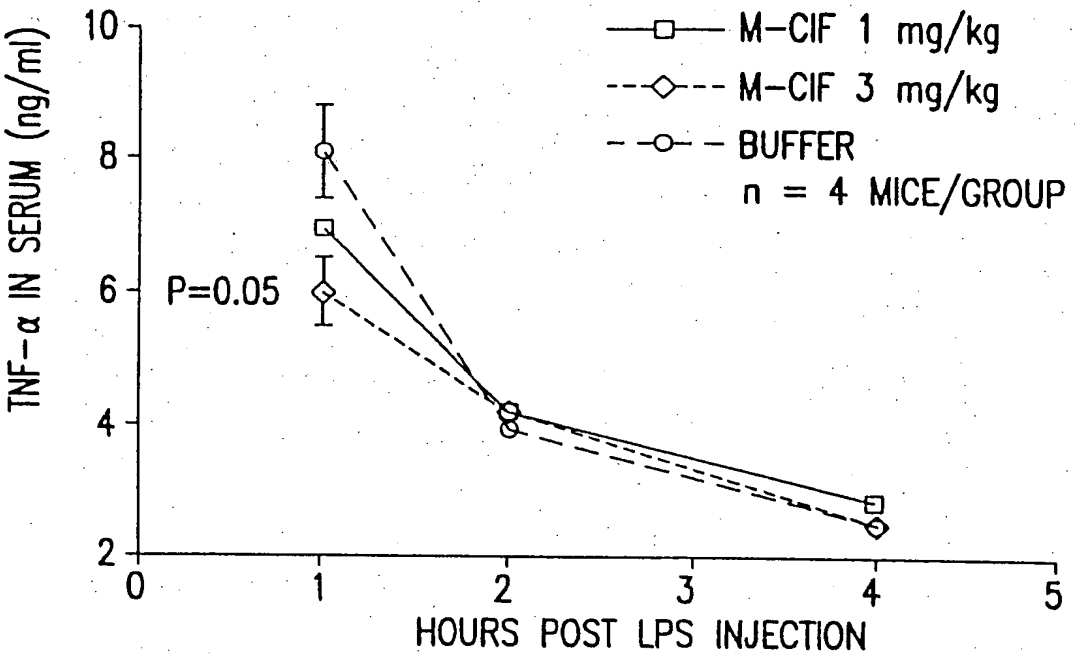

TNF-α IN SERUM (ng/ml)
10
8
6
4
2
0
1
2
3
4
5
HOURS POST LPS INJECTION
P=0.05
M-CIF 1 mg/kg
M-CIF 3 mg/kg
BUFFER
n = 4 MICE/GROUP

FIG.43

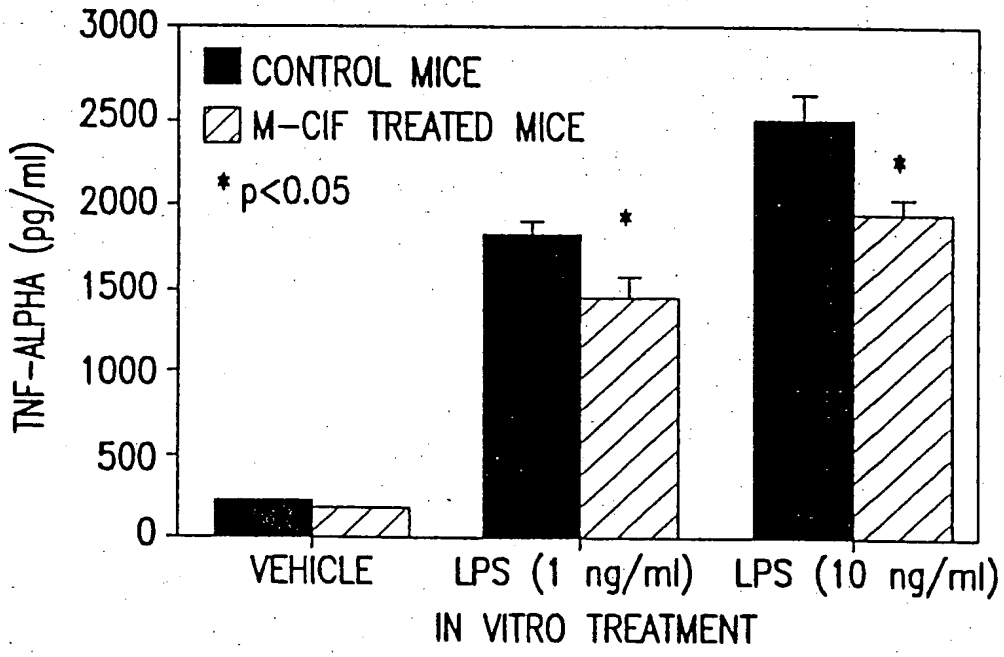
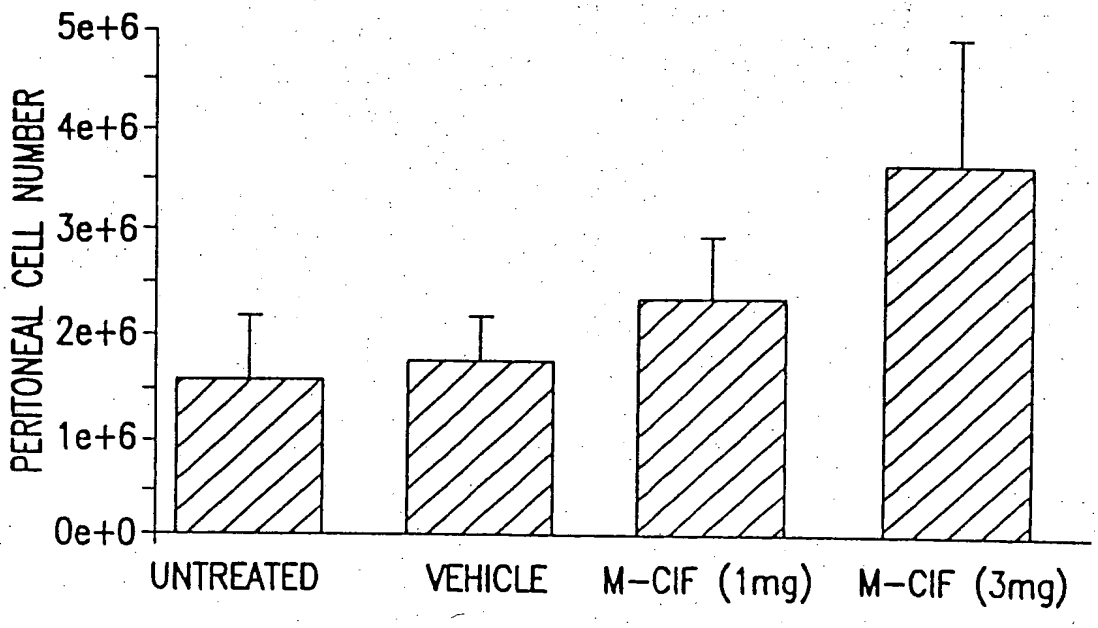

3000
2500
2000
1500
1000
500
0
TNF-ALPHA (pg/ml)
CONTROL MICE
M-CIF TREATED MICE
* p<0.05
*
*
VEHICLE
LPS (1 ng/ml)
LPS (10 ng/ml)
IN VITRO TREATMENT

FIG.44



UNTREATED	VEHICLE	M-CIF (1mg/kg)	M-CIF (3mg/kg)	
UNTREATED		1mg/kg		p=0.019
	VEHICLE	1mg/kg		p=0.027
UNTREATED			3mg/kg	p=0.0003
	VEHICLE		3mg/kg	p=0.0003
		1mg/kg	3mg/kg	p=0.007

FIG.45

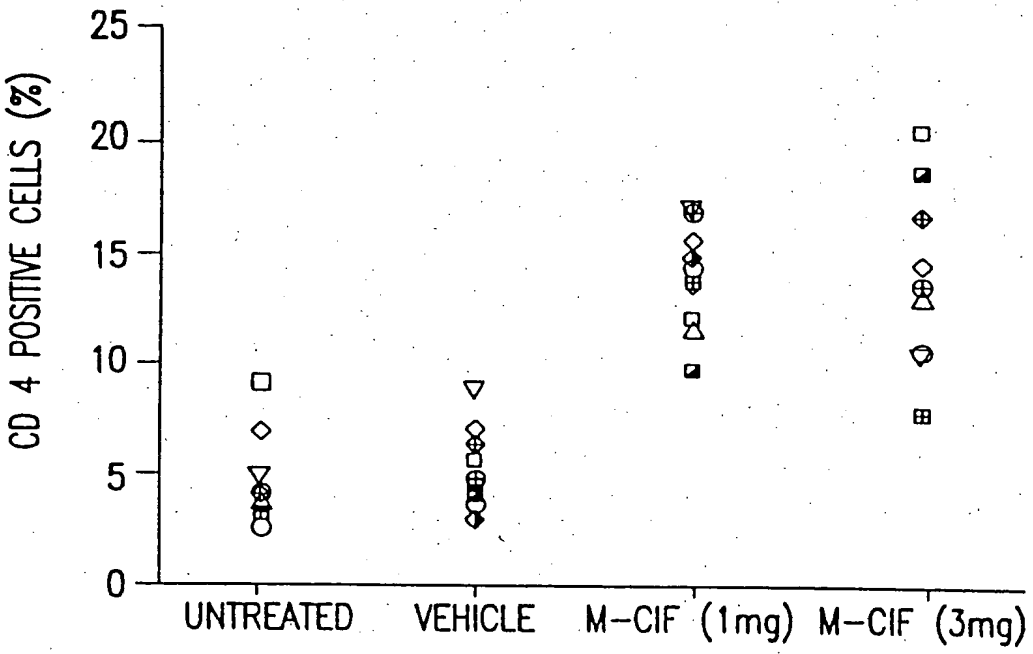

CD 4 POSITIVE CELLS (%)
25
20
15
10
5
0
UNTREATED
VEHICLE
M-CIF (1mg)
M-CIF (3mg)

FIG.46

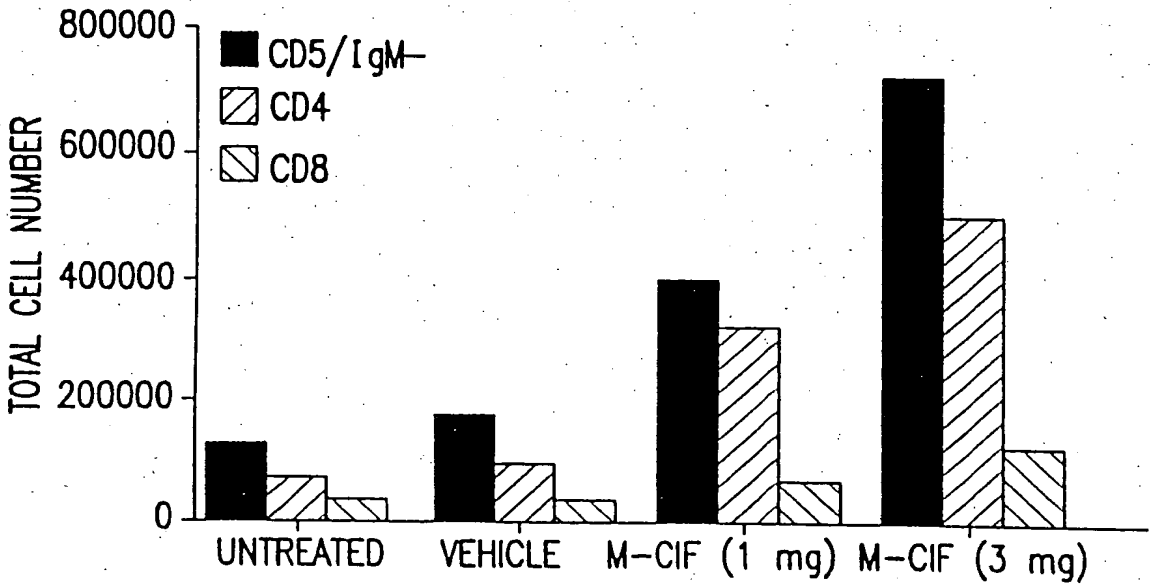

800000
600000
400000
200000
0
TOTAL CELL NUMBER
CD5/IgM-
CD4
CD8
UNTREATED
VEHICLE
M-CIF (1 mg)
M-CIF (3 mg)

FIG.47

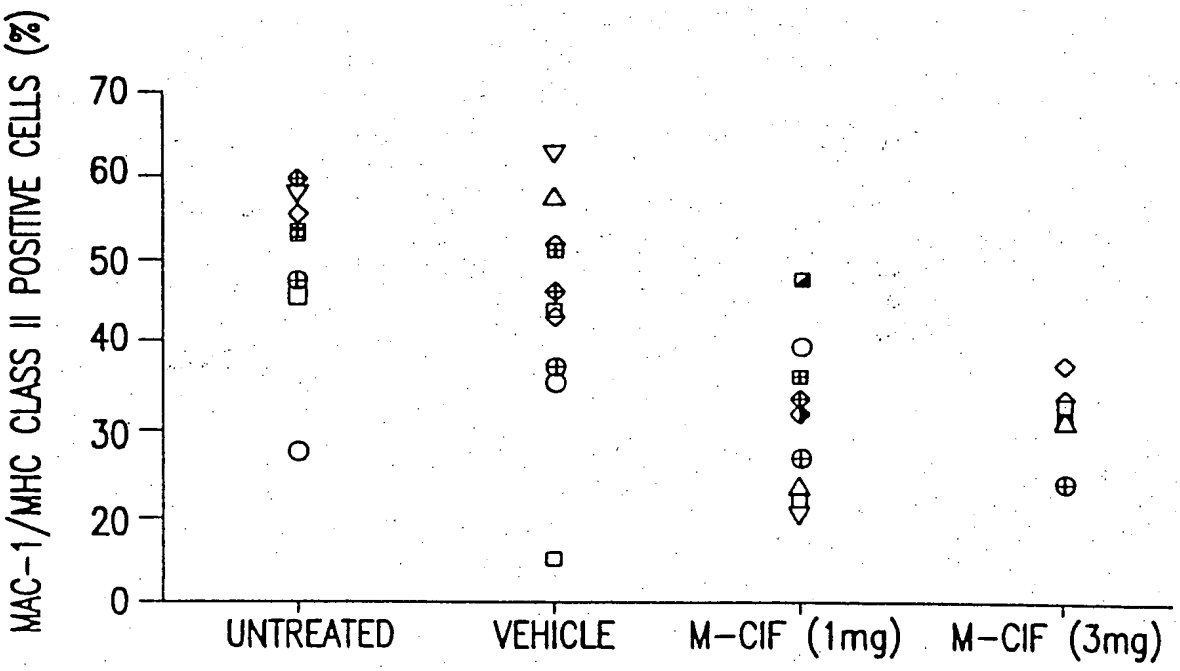


FIG.48A

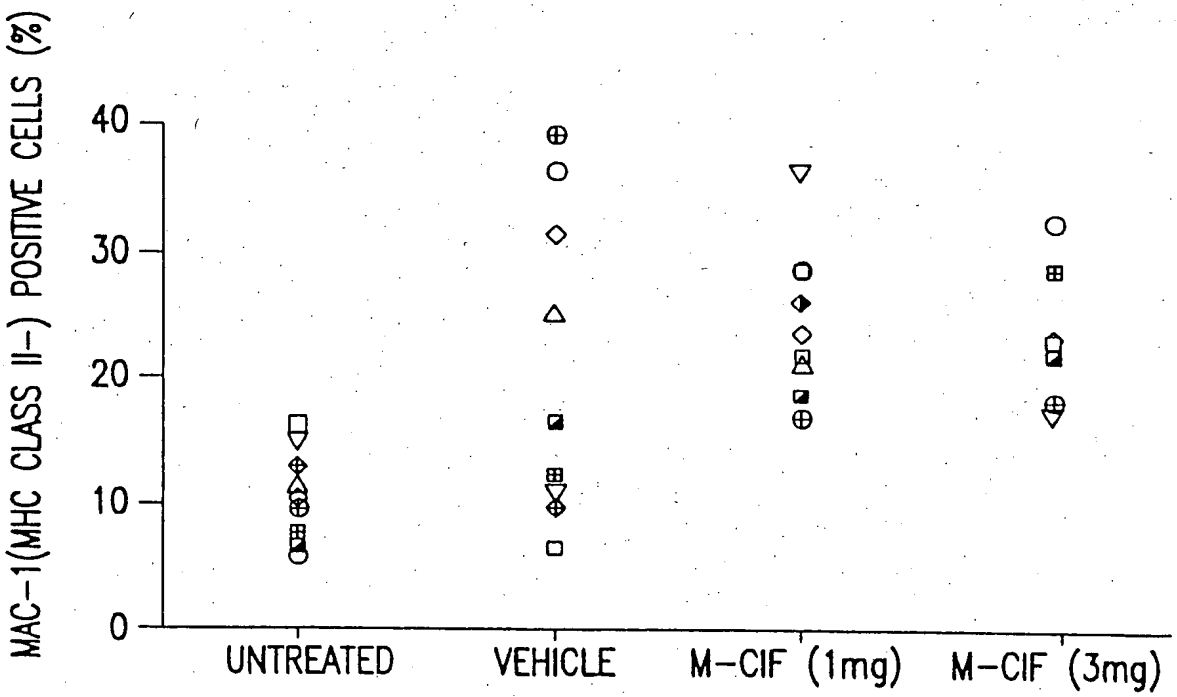


FIG.48B

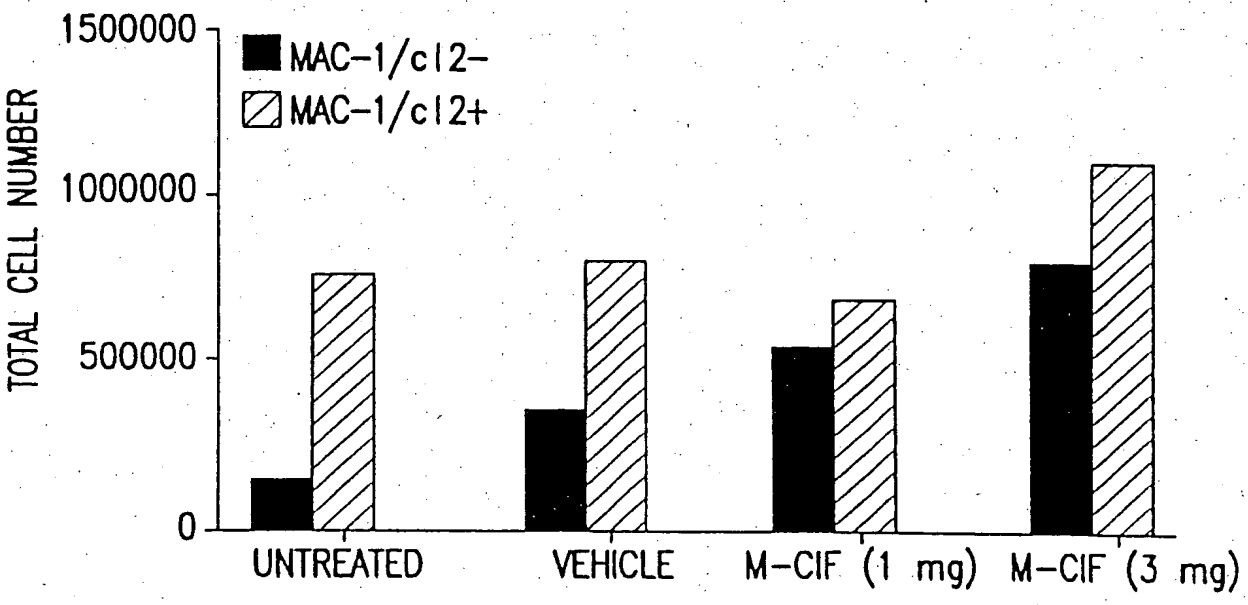
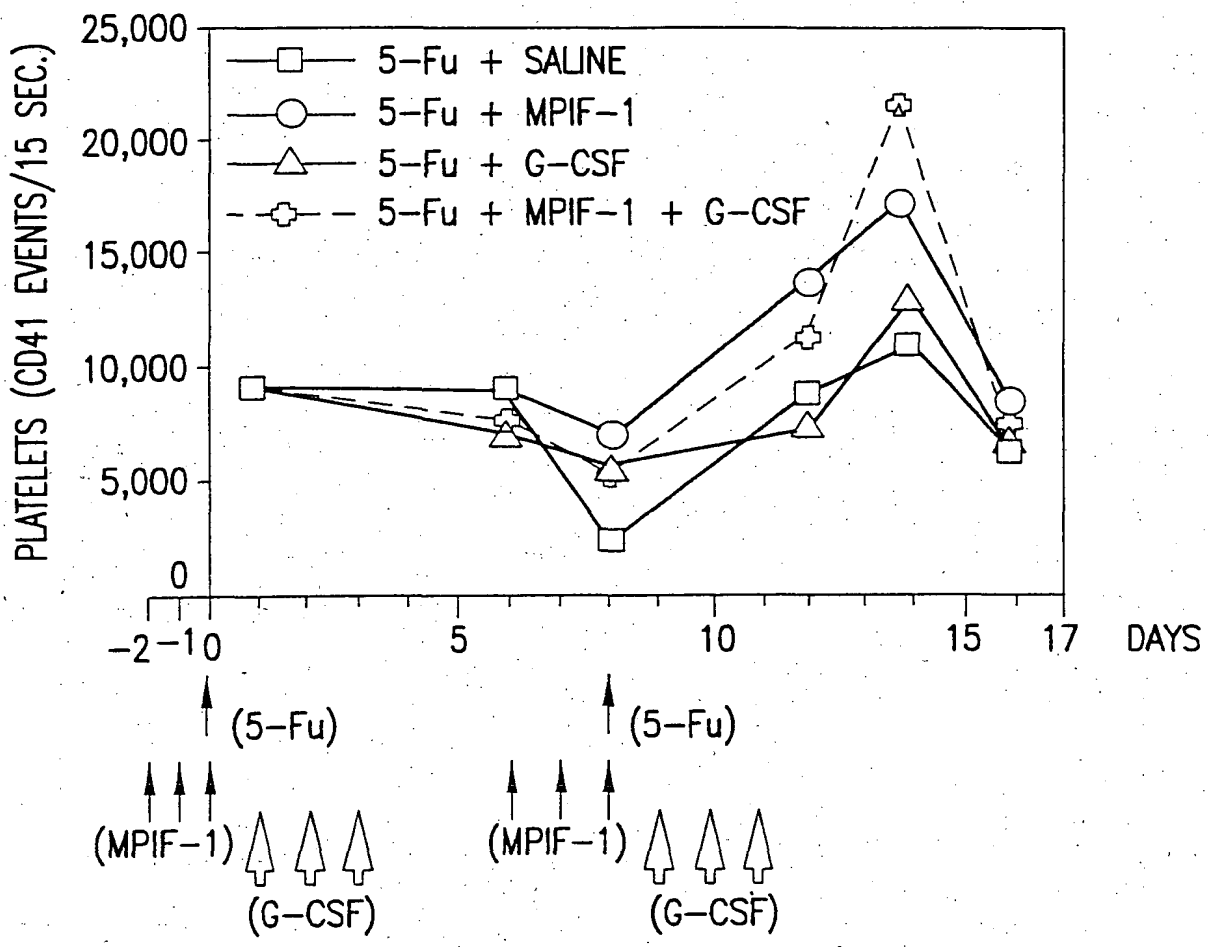

1500000
1000000
500000
0
TOTAL CELL NUMBER
MAC-1/c12-
MAC-1/c12+
UNTREATED
VEHICLE
M-CIF (1 mg)
M-CIF (3 mg)

FIG.49

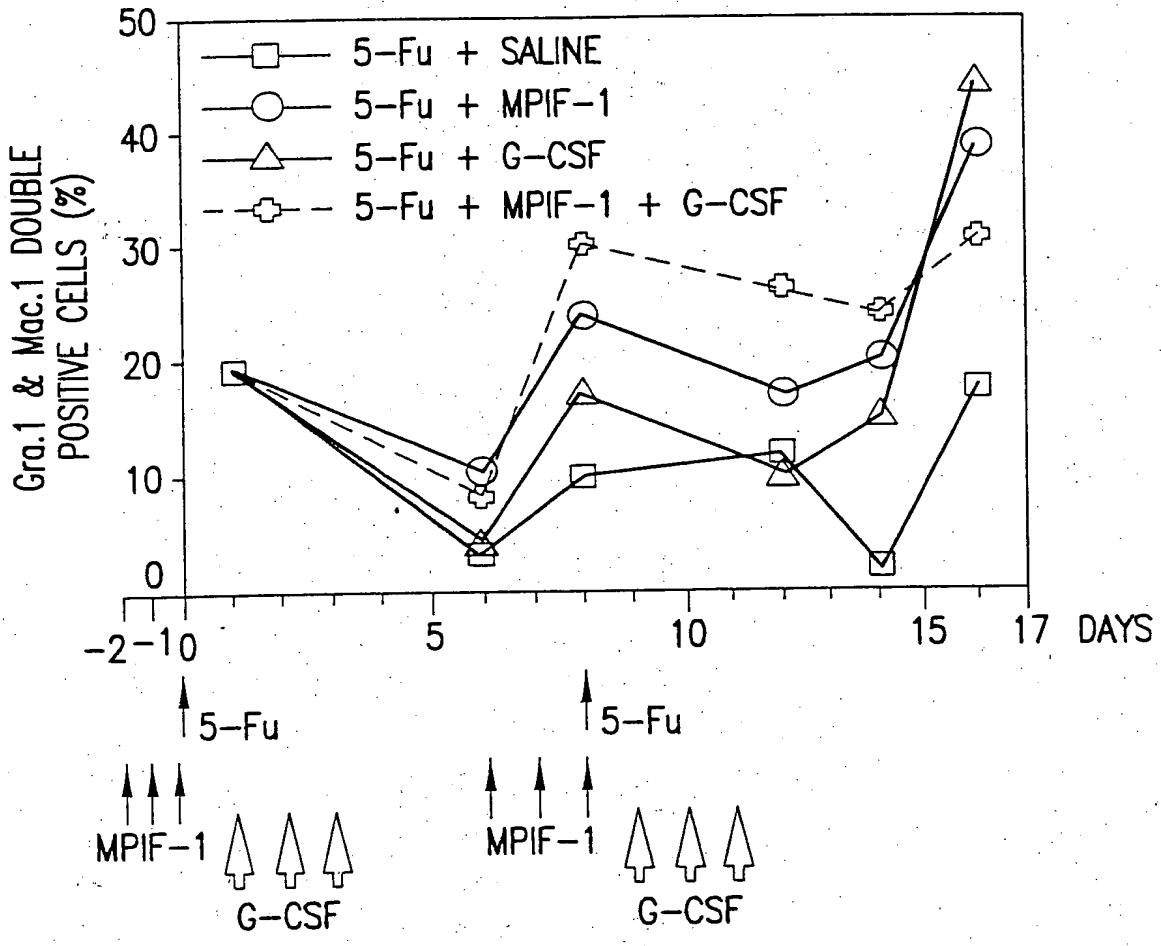
STEM CELL MOBILIZATION IN RESPONSE TO ADMINISTERING MPIF-1 TO NORMAL MICE				
EXPERIMENT	TREATMENTS	WBC/ml BLOOD (x 10^6)	PHENOTYPE OF CELLS	
			Gr.1	$CD34^+Sca\text{-}1^+$
1.	SALINE	4.7 ± 0.36	10	0.20
	MPIF-1	7.1 ± 0.63	39	8

FIG.50



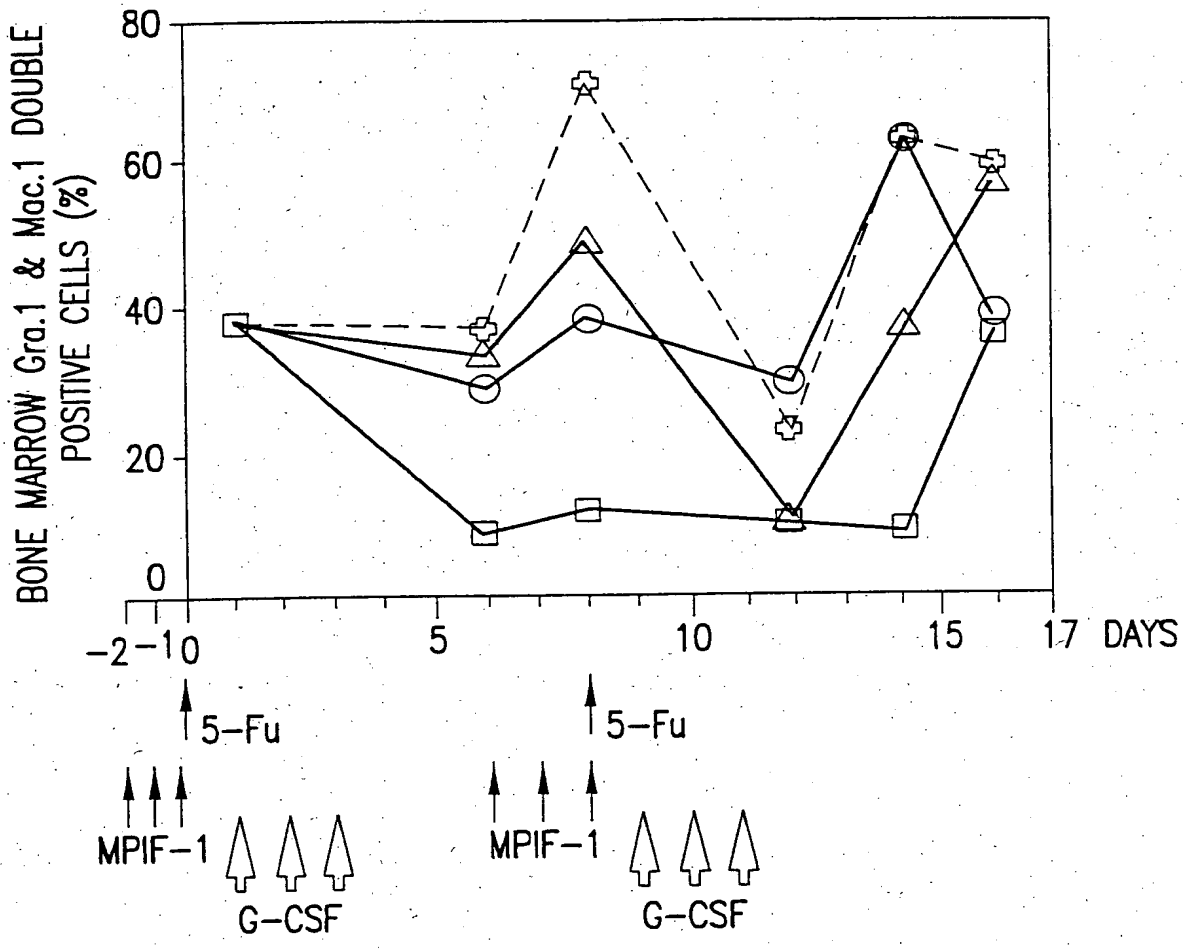
5-Fu: I.P. INJECTION, 100 mg/Kg
MPIF-1: I.P. INJECTION, 1.0 mg/Kg
G-CSF: I.P. INJECTION, 0.5 mg/Kg

FIG.51



5-Fu: I.P. INJECTION, 100 mg/Kg
MPIF-1: I.P. INJECTION, 1.0 mg/Kg
G-CSF: I.P. INJECTION, 0.5 mg/Kg

FIG.52



5-Fu: I.P. INJECTION, 100 mg/Kg
MPIF-1: I.P. INJECTION, 1.0 mg/Kg
G-CSF: I.P. INJECTION, 0.5 mg/Kg

FIG.53

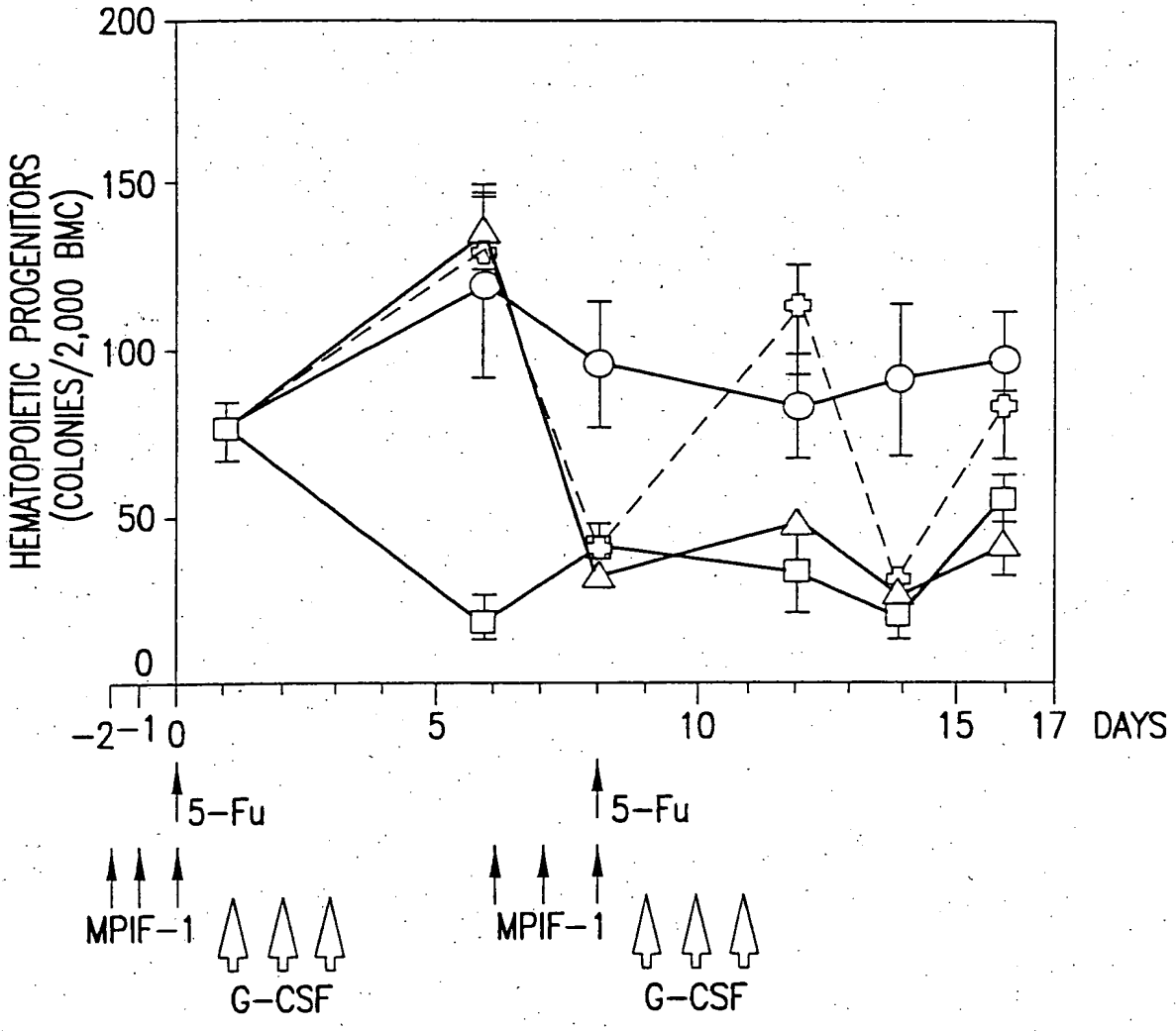

HEMATOPOIETIC PROGENITORS
(COLONIES/2,000 BMC)
200
150
100
50
0
-2
-1
0
5
10
15
17
DAYS
5-Fu
5-Fu
MPIF-1
MPIF-1
G-CSF
G-CSF
5-Fu + SALINE
5-Fu + MPIF-1
5-Fu + G-CSF
5-Fu + MPIF-1 + G-CSF
5-Fu: I.P. INJECTION, 100 mg/Kg
MPIF-1: I.P. INJECTION, 1.0 mg/Kg
G-CSF: I.P. INJECTION, 0.5 mg/Kg

FIG.54

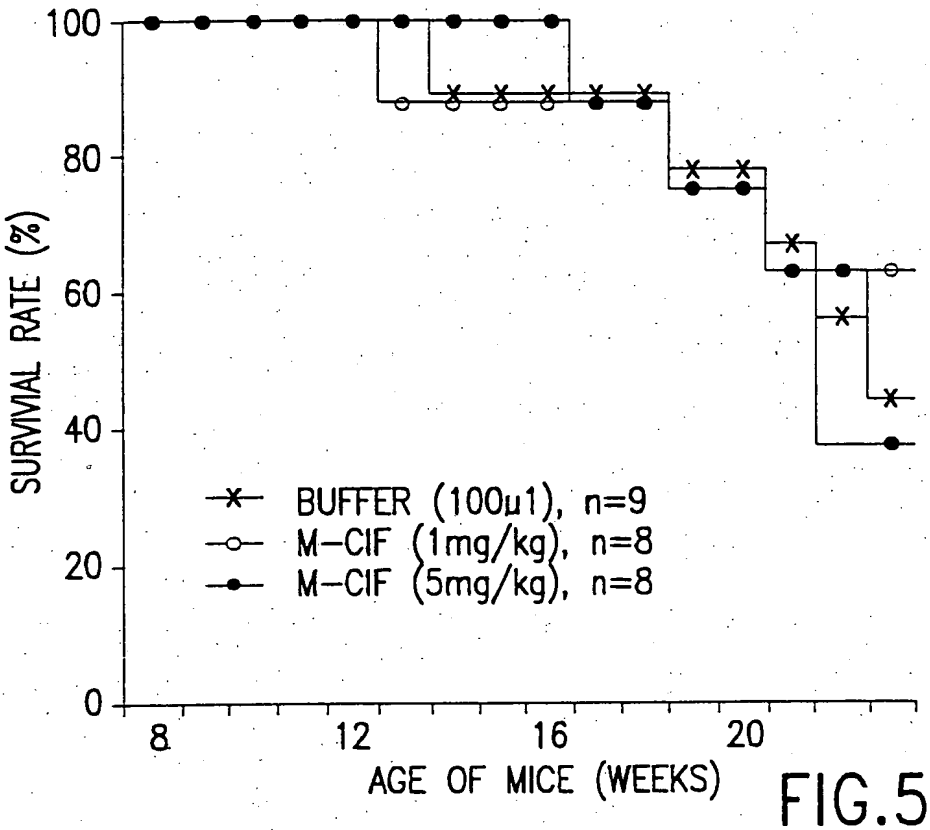

100
80
60
40
20
0
SURVIVIAL RATE (%)
BUFFER (100µl), n=9
M-CIF (1mg/kg), n=8
M-CIF (5mg/kg), n=8
8
12
16
20
AGE OF MICE (WEEKS)

FIG.55

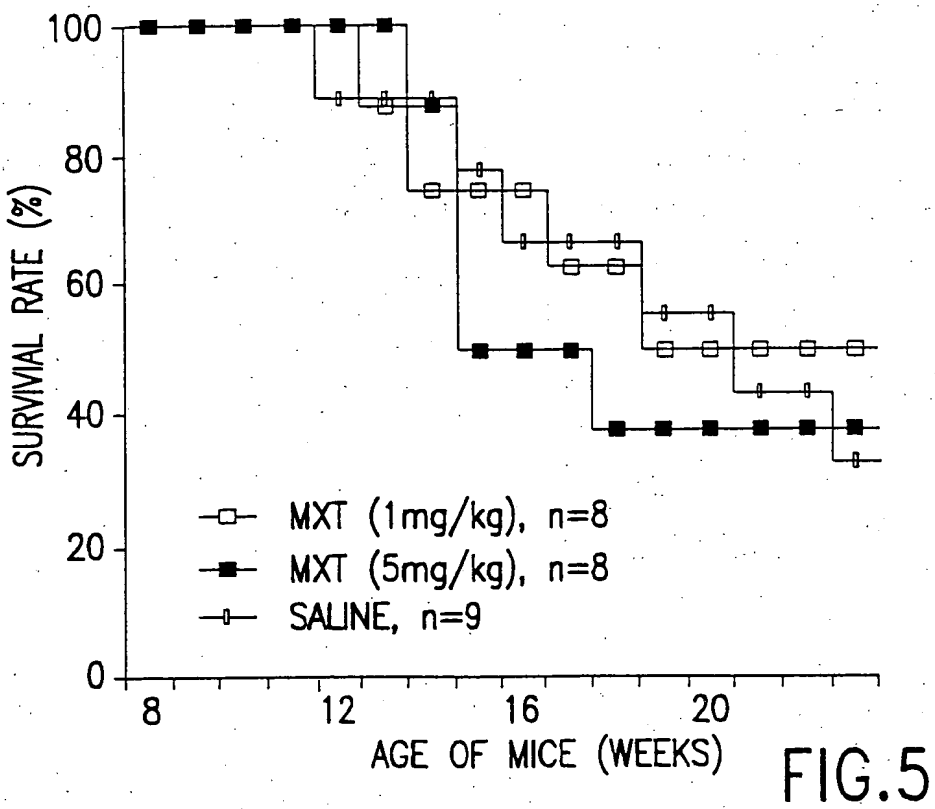

100
80
60
40
20
0
SURVIVIAL RATE (%)
MXT (1mg/kg), n=8
MXT (5mg/kg), n=8
SALINE, n=9
8
12
16
20
AGE OF MICE (WEEKS)

FIG.56

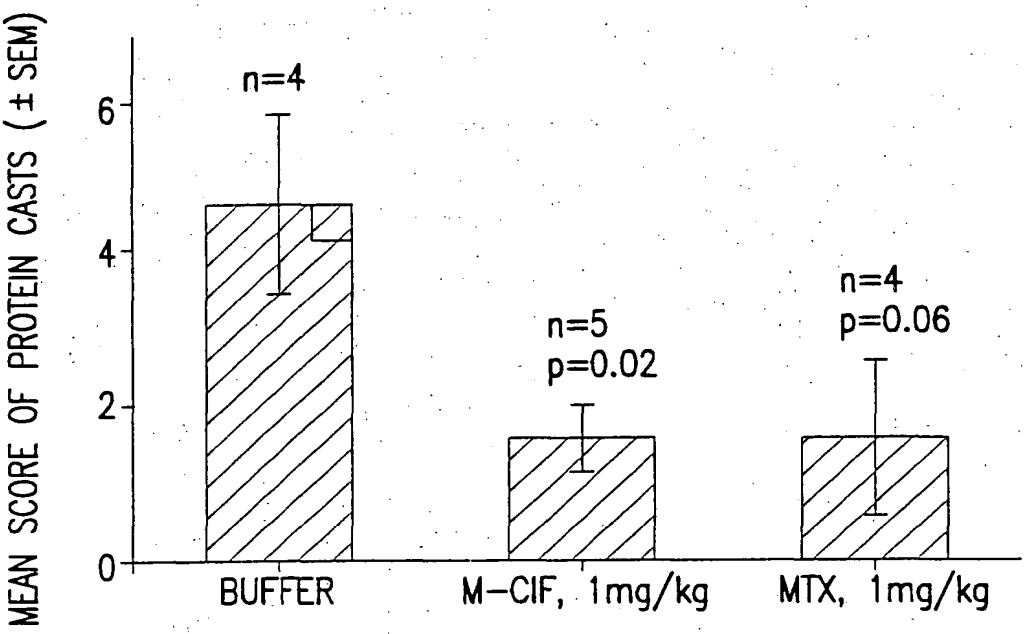

MEAN SCORE OF PROTEIN CASTS (± SEM)
0
2
4
6
n=4
n=5
p=0.02
n=4
p=0.06
BUFFER
M-CIF, 1mg/kg
MTX, 1mg/kg

FIG.57

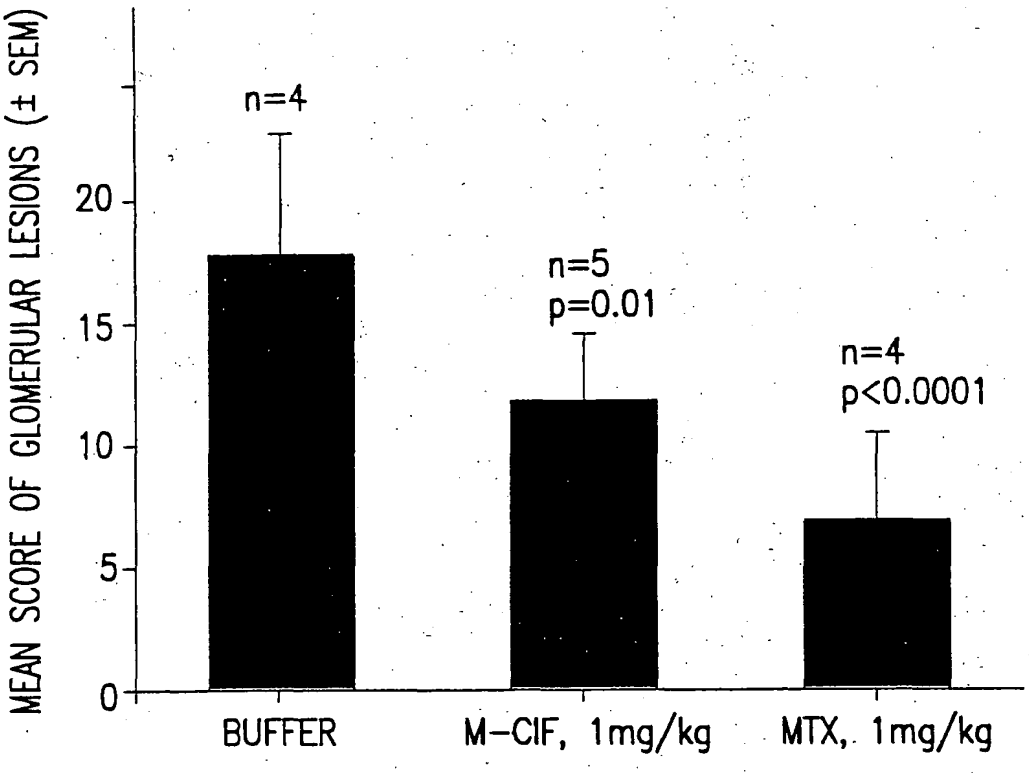

MEAN SCORE OF GLOMERULAR LESIONS (± SEM)
0
5
10
15
20
n=4
n=5
p=0.01
n=4
p<0.0001
BUFFER
M-CIF, 1mg/kg
MTX, 1mg/kg

FIG.58

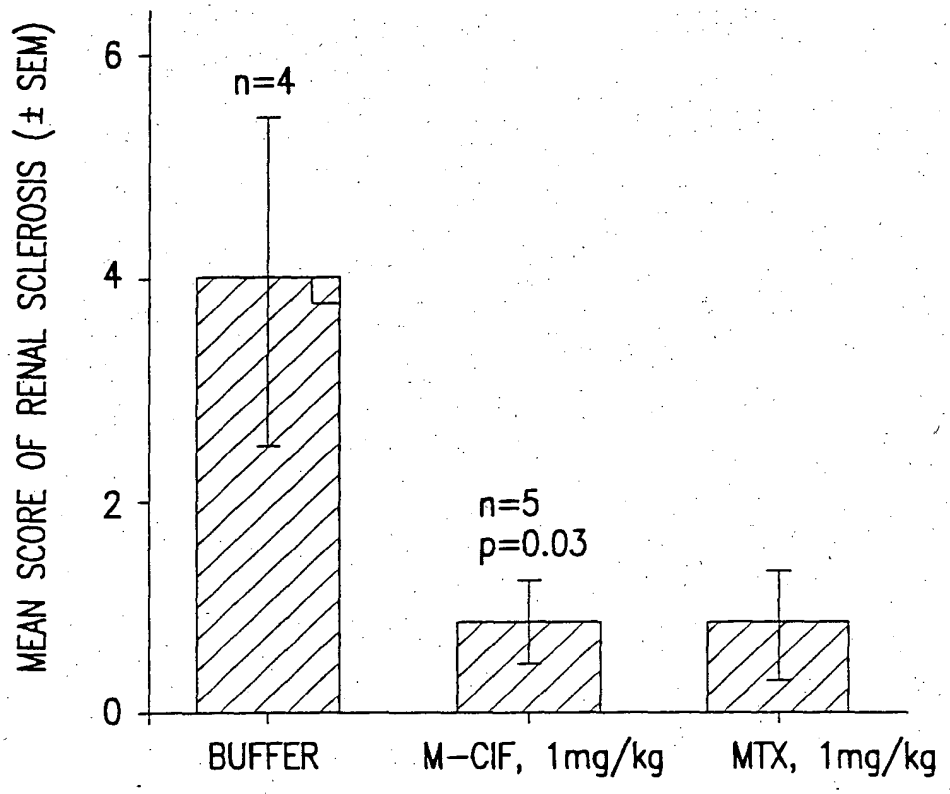

MEAN SCORE OF RENAL SCLEROSIS (± SEM)
6
4
2
0
n=4
n=5
p=0.03
BUFFER
M-CIF, 1mg/kg
MTX, 1mg/kg

FIG.59

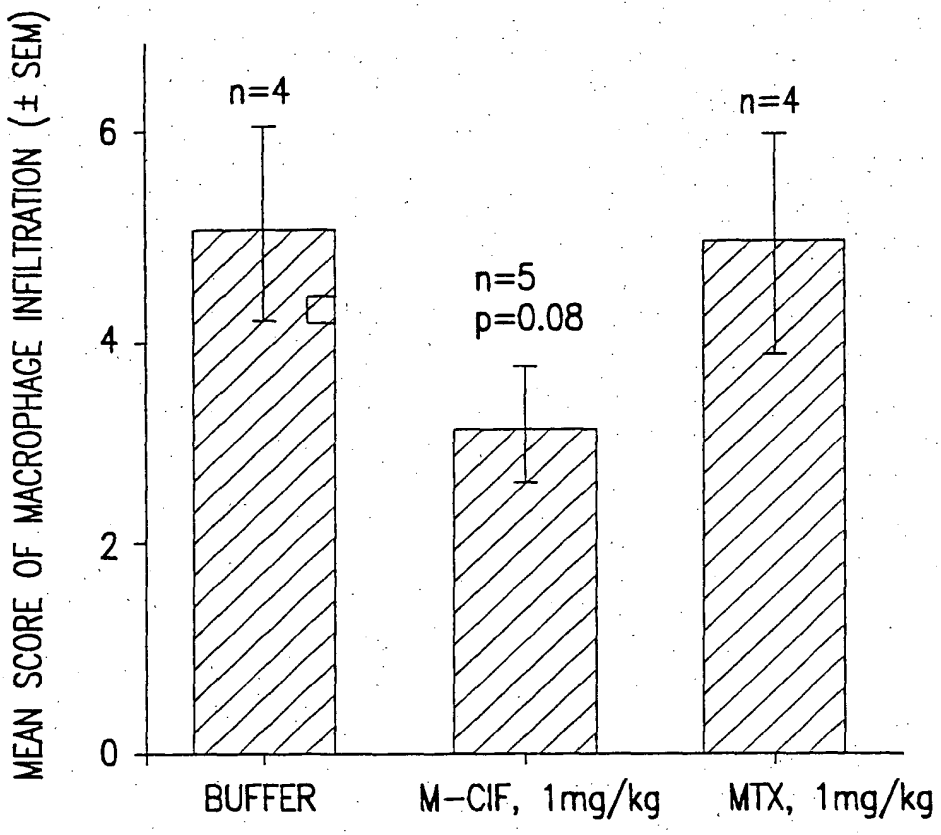

MEAN SCORE OF MACROPHAGE INFILTRATION (± SEM)
6
4
2
0
n=4
n=5
p=0.08
n=4
BUFFER
M-CIF, 1mg/kg
MTX, 1mg/kg

FIG.60

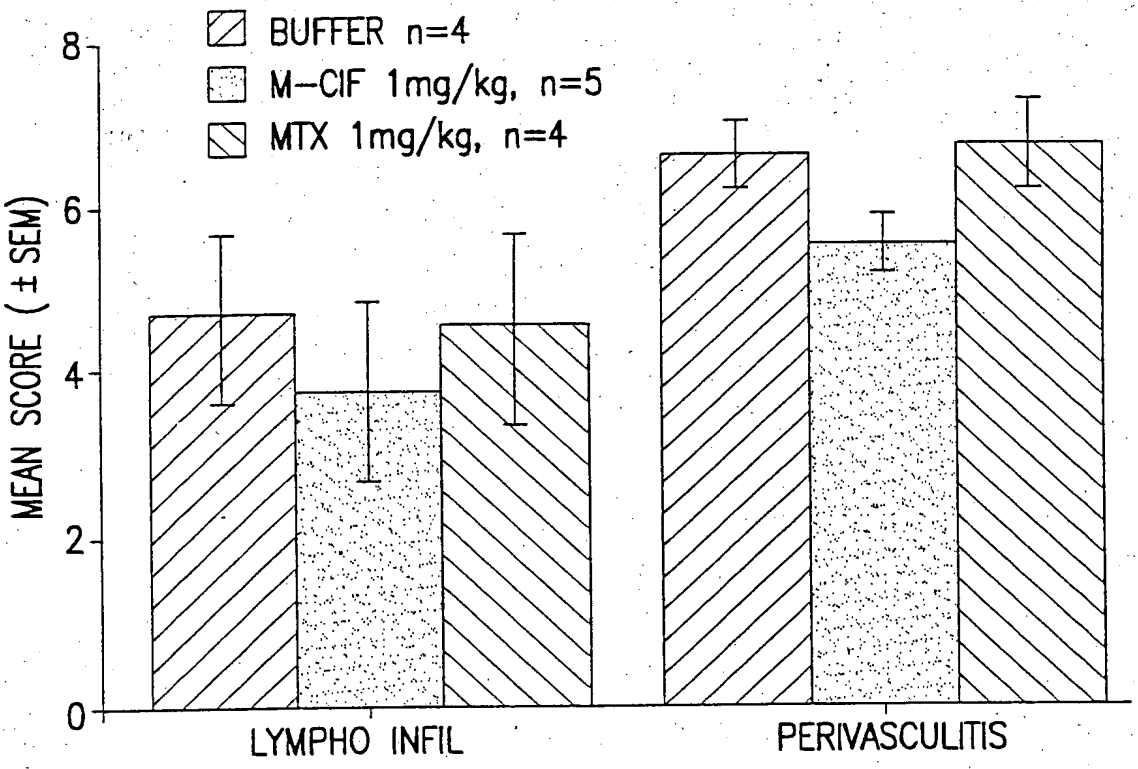

BUFFER n=4
M-CIF 1mg/kg, n=5
MTX 1mg/kg, n=4
MEAN SCORE (± SEM)
8
6
4
2
0
LYMPHO INFIL
PERIVASCULITIS

FIG.61

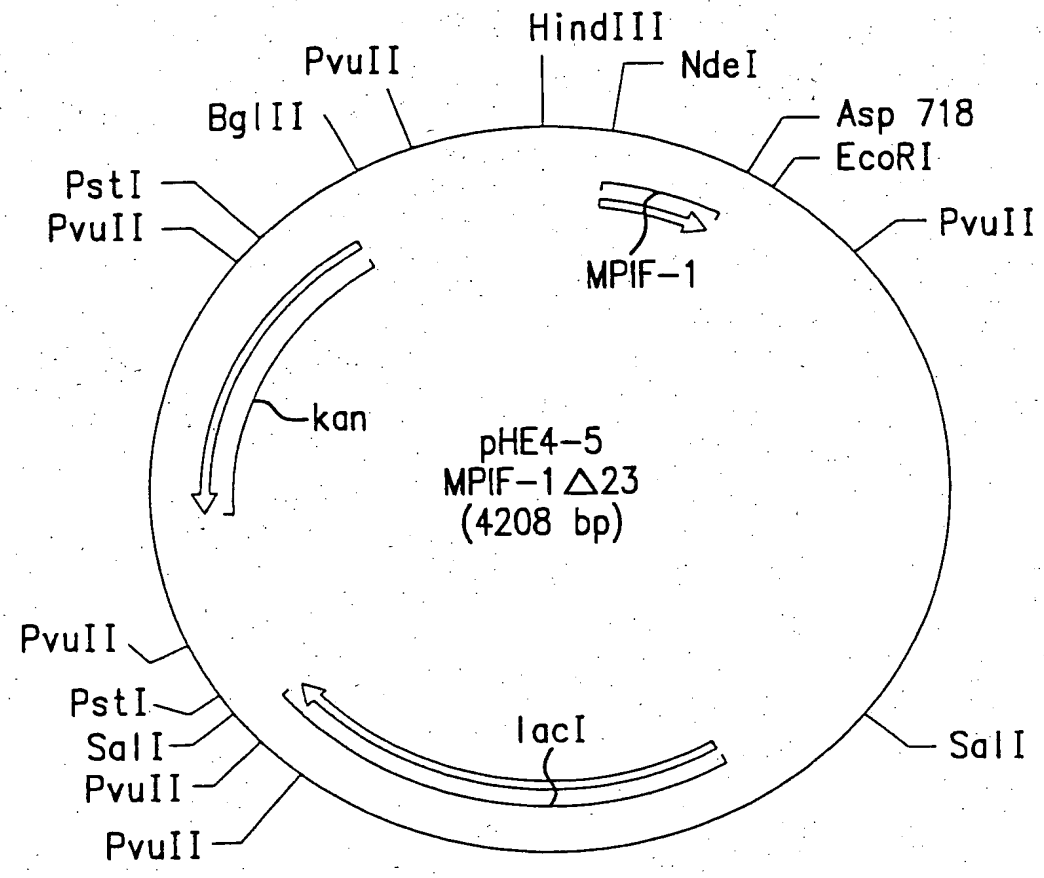

HindIII
NdeI
PvuII
BglII
Asp 718
EcoRI
PstI
PvuII
PvuII
MPIF-1
kan
pHE4-5
MPIF-1△23
(4208 bp)
PvuII
PstI
SalI
PvuII
PvuII
lacI
SalI

FIG.62

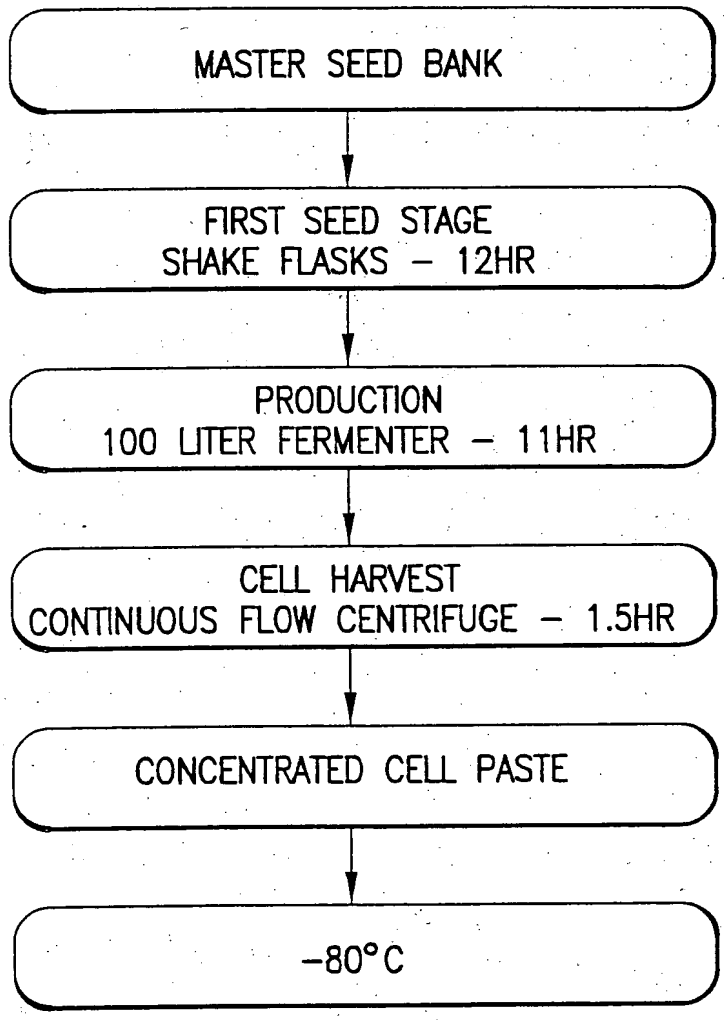

MASTER SEED BANK
FIRST SEED STAGE
SHAKE FLASKS – 12HR
PRODUCTION
100 LITER FERMENTER – 11HR
CELL HARVEST
CONTINUOUS FLOW CENTRIFUGE – 1.5HR
CONCENTRATED CELL PASTE
–80°C

FIG.63

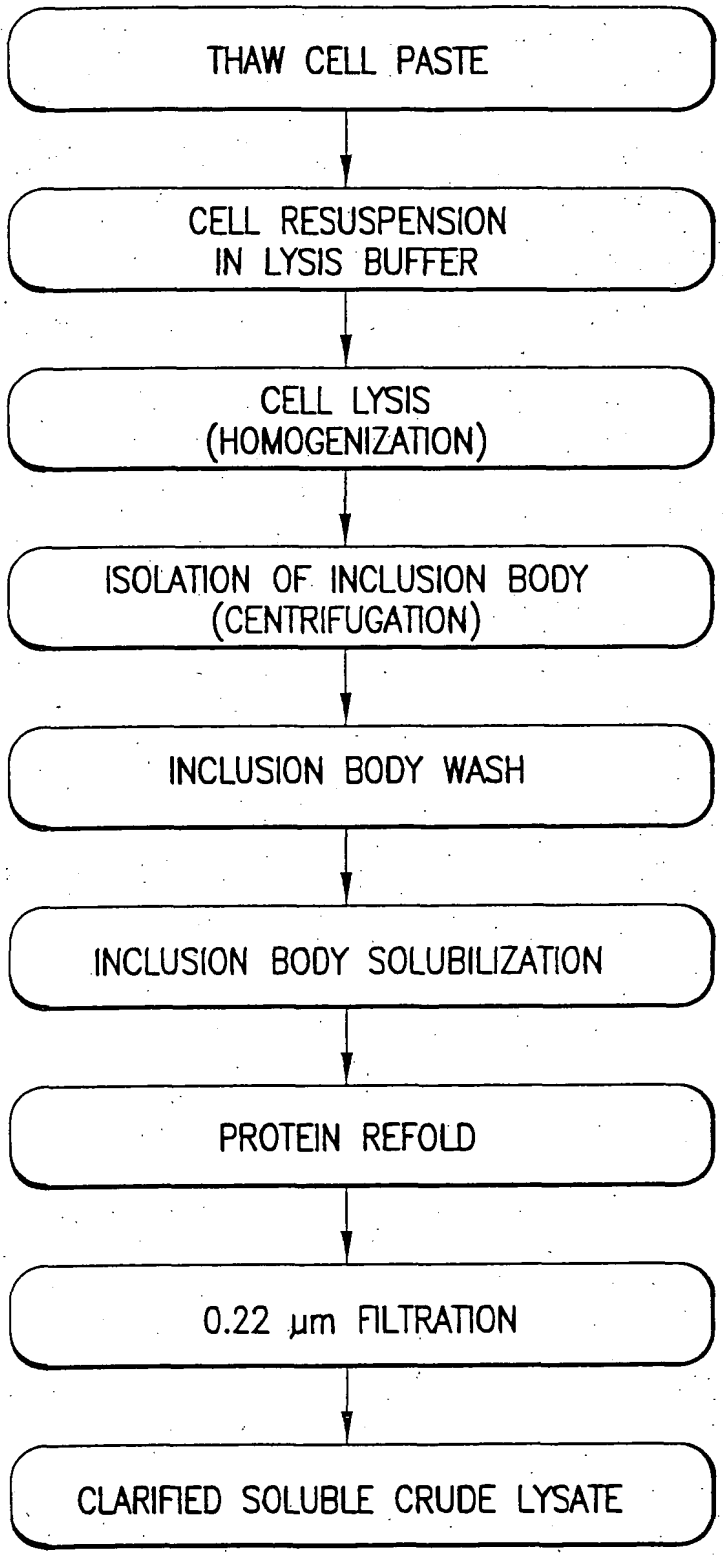

THAW CELL PASTE
CELL RESUSPENSION IN LYSIS BUFFER
CELL LYSIS (HOMOGENIZATION)
ISOLATION OF INCLUSION BODY (CENTRIFUGATION)
INCLUSION BODY WASH
INCLUSION BODY SOLUBILIZATION
PROTEIN REFOLD
0.22 µm FILTRATION
CLARIFIED SOLUBLE CRUDE LYSATE

FIG.64

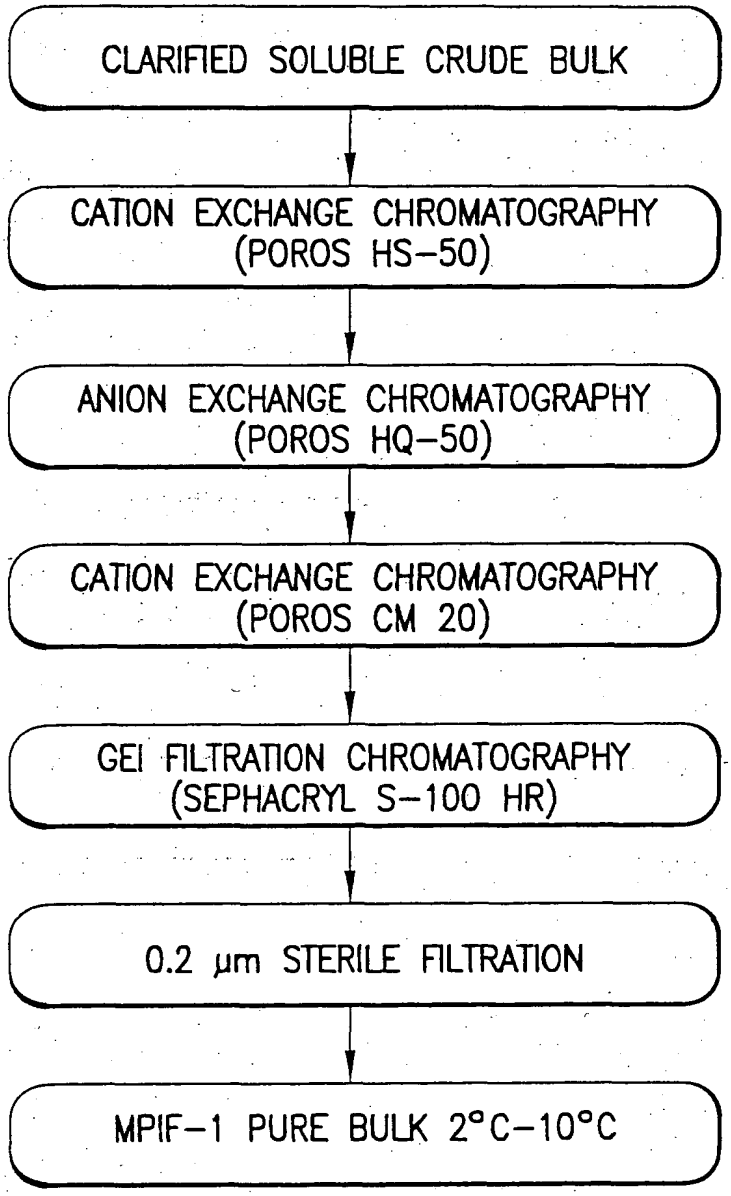

CLARIFIED SOLUBLE CRUDE BULK
CATION EXCHANGE CHROMATOGRAPHY (POROS HS-50)
ANION EXCHANGE CHROMATOGRAPHY (POROS HQ-50)
CATION EXCHANGE CHROMATOGRAPHY (POROS CM 20)
GEI FILTRATION CHROMATOGRAPHY (SEPHACRYL S-100 HR)
0.2 μm STERILE FILTRATION
MPIF-1 PURE BULK 2°C-10°C

FIG.65

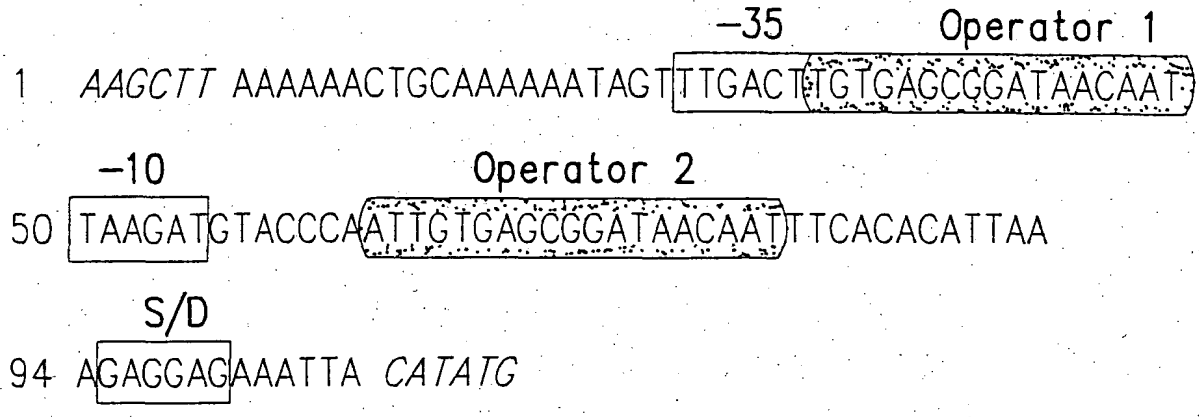

−35
Operator 1
1 AAGCTT AAAAAACTGCAAAAAATAGTTTGACTTGTGAGCGGATAACAAT
−10
Operator 2
50 TAAGATGTACCCAATTGTGAGCGGATAACAATTTCACACATTAA
S/D
94 AGAGGAGAAATTA CATATG

FIG.66

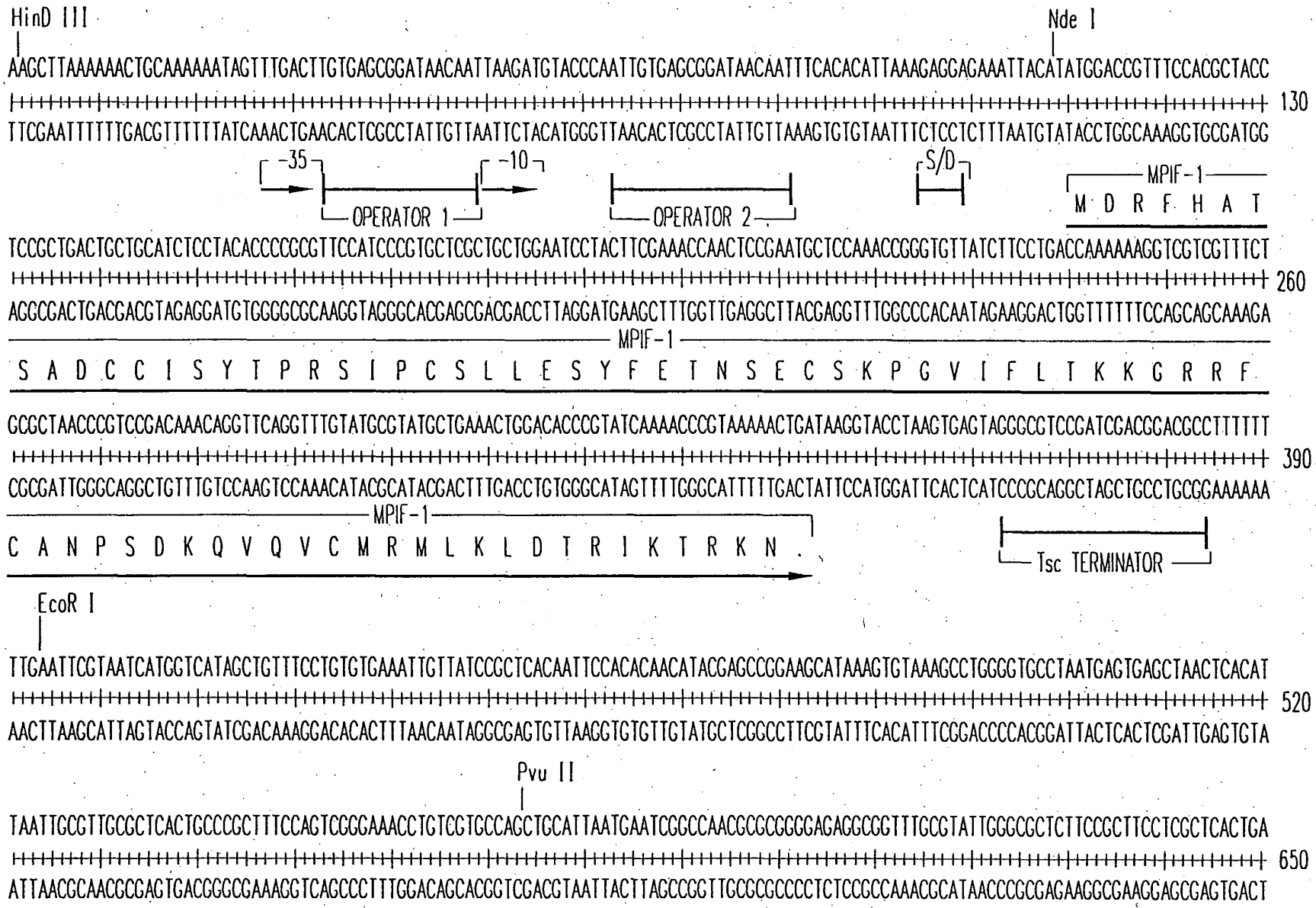

HinD III
Nde I
AAGCTTAAAAAACTGCAAAAAATAGTTTGACTTGTGAGCGGATAACAATTAAGATGTACCCAATTGTGAGCGGATAACAATTTCACACATTAAAGAGGAGAAATTACATATGGACCGTTTCCACGCTACC
130
TTCGAATTTTTTGACGTTTTTTATCAAACTGAACACTCGCCTATTGTTAATTCTACATGGGTTAACACTCGCCTATTGTTAAAGTGTGTAATTTCTCCTCTTTAATGTATACCTGGCAAAGGTGCGATGG
-35
-10
OPERATOR 1
OPERATOR 2
S/D
MPIF-1
M D R F H A T
TCCGCTGACTGCTGCATCTCCTACACCCCGCGTTCCATCCCGTGCTCGCTGCTGGAATCCTACTTCGAAACCAACTCCGAATGCTCCAAACCGGGTGTTATCTTCCTGACCAAAAAAGGTCGTCGTTTCT
260
AGGCGACTGACGACGTAGAGGATGTGGGCGCGCAAGGTAGGGCACGAGCGACGACCTTAGGATGAAGCTTTGGTTGAGGCTTACGAGGTTTGGCCCACAATAGAAGGACTGGTTTTTTCCAGCAGCAAAGA
MPIF-1
S A D C C I S Y T P R S I P C S L L E S Y F E T N S E C S K P G V I F L T K K G R R F
GCGCTAACCCGTCCGACAAACAGGTTCAGGTTTGTATGCGTATGCTGAAACTGGACACCCGTATCAAAACCCGTAAAAACTGATAAGGTACCTAAGTGAGTAGGGCGTCCGATCGACGGACGCCTTTTTT
390
CGCGATTGGGCAGGCTGTTTGTCCAAGTCCAAACATACGCATACGACTTTGACCTGTGGGCATAGTTTTGGGCATTTTTGACTATTCCATGGATTCACTCATCCCGCAGGCTAGCTGCCTGCGGAAAAAA
MPIF-1
C A N P S D K Q V Q V C M R M L K L D T R I K T R K N .
Tsc TERMINATOR
EcoR I
TTGAATTCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACAT
520
AACTTAAGCATTAGTACCAGTATCGACAAAGGACACACTTTAACAATAGGCGAGTGTTAAGGTGTGTTGTATGCTCGGCCTTCGTATTTCACATTTCGGACCCCACGGATTACTCACTCGATTGAGTGTA
Pvu II
TAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGA
650
ATTAACGCAACGCGAGTGACGGGCGAAAGGTCAGCCCTTTGGACAGCACGGTCGACGTAATTACTTAGCCGGTTGCGCGCCCCTCTCCGCCAAACGCATAACCCGCGAGAAGGCGAAGGAGCGAGTGACT

FIG.67A

CTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGG
780
GAGCGACGCGAGCCAGCAAGCCGACGCCGCTCGCCATAGTCGAGTGAGTTTCCGCCATTATGCCAATAGGTGTCTTAGTCCCCTATTGCGTCCTTTCTTGTACACTCGTTTTCCGGTCGTTTTCCGGTCC
AACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCC
910
TTGGCATTTTTCCGGCGCAACGACCGCAAAAAGGTATCCGAGGCGGGGGGACTGCTCGTAGTGTTTTTAGCTGCGAGTTCAGTCTCCACCCCTTTGGGCTGTCCTGATATTTCTATGGTCCGCAAAGGGG
CTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGT
1040
GACCTTCGAGGGAGCACGCGAGAGGACAAGGCTGCGACGGCGAATGGCCTATGGACAGGCGGAAAGACGGAAGCCCTTCGCACCGCGAAAGAGTATCGAGTGCGACATCCATAGAGTCAAGCCACATCCA
oriC
CGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGT
1170
GCAAGCGAGGTTCGACCCGACACACGTGCTTGGGGGGCAAGTCGGGCTGGCGACGCGGAATAGGCCATTGATAGCAGAACTCAGGTTGGGCCATTCTGTGCTGAATAGCGGTGACCGTCGTCGGTGACCA
AACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAA
1300
TTGTCCTAATCGTCTCGCTCCATACATCCGCCACGATGTCTCAAGAACTTCACCACCGGATTGATGCCGATGTGATCTTCTTGTCATAAACCATAGACGCGAGACGACTTCCGTCAATGGAAGCCTTTTT
GAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGA
1430
CTCAACCATCGAGAACTAGGCCGTTTGTTTGGTGGCGACCATCGCCACCAAAAAAACAAACGTTCGTCGTCTAATGCGCGTCTTTTTTTCCTAGAGTTCTTCTAGGAAACTAGAAAAGATGCCCCAGACT
SaI I
CGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCGTCGACAATTCGCGCGCGAAGGCGAAGCGGCATGCATTTACGTTGACACCATCGAATGGTGCAAAACCTTTCGCGGTAT
1560
GCGAGTCACCTTGCTTTTGAGTGCAATTCCCTAAAACCAGTACTCTAATAGCAGCTGTTAAGCGCGCGCTTCCGCTTCGCCGTACGTAAATGCAACTGTGGTAGCTTACCACGTTTTGGAAAGCGCCATA

FIG.67B

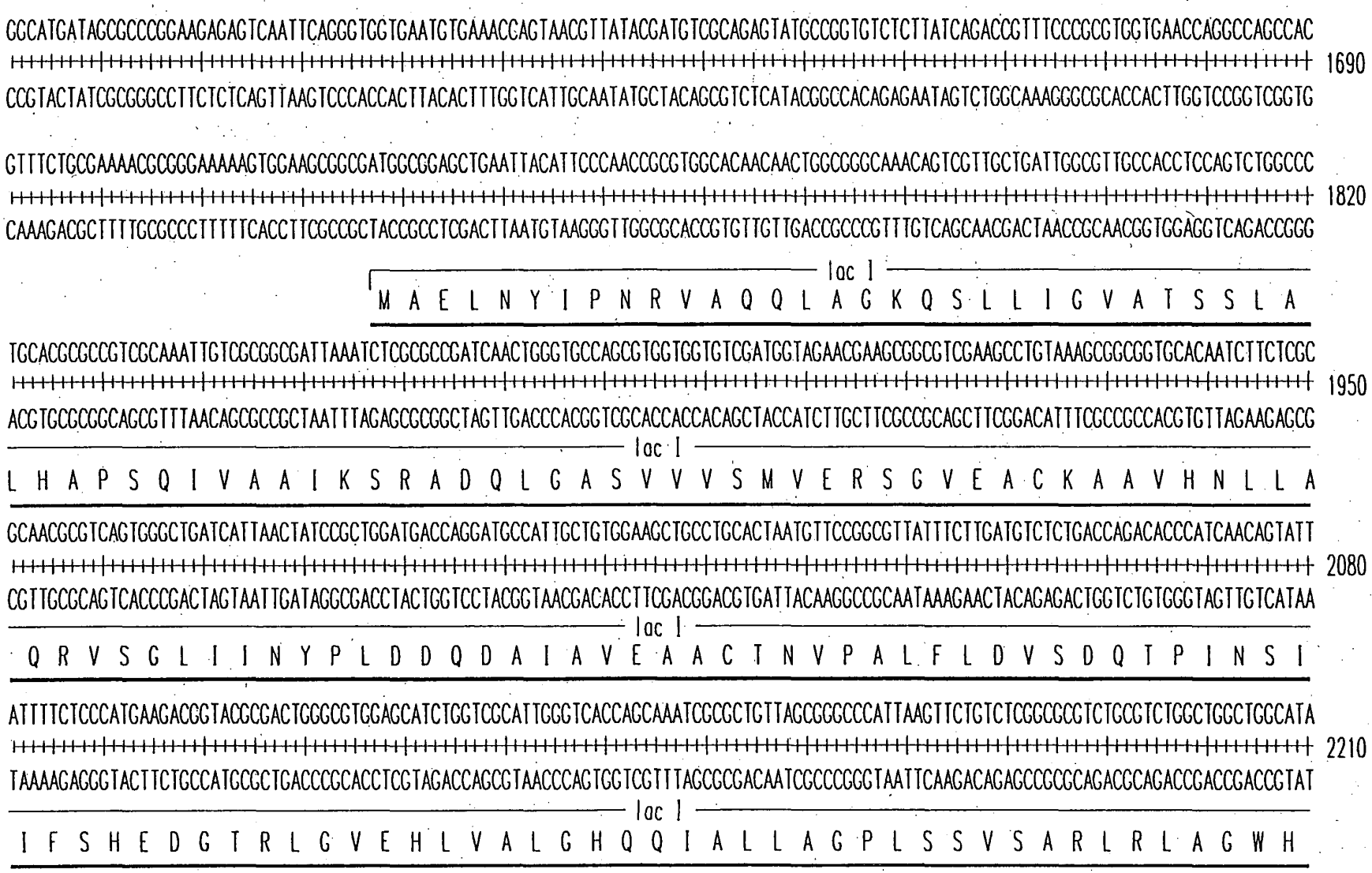

GGCATGATAGCGCCCGGAAGAGAGTCAATTCAGGGTGGTGAATGTGAAACCAGTAACGTTATACGATGTCGCAGAGTATGCCGGTGTCTCTTATCAGACCGTTTCCCGCGTGGTGAACCAGGCCAGCCAC
1690
CCGTACTATCGCGGGCCTTCTCTCAGTTAAGTCCCACCACTTACACTTTGGTCATTGCAATATGCTACAGCGTCTCATACGGCCACAGAGAATAGTCTGGCAAAGGGCGCACCACTTGGTCCGGTCGGTG
GTTTCTGCGAAAACGCGGGAAAAAGTGGAAGCGGCGATGGCGGAGCTGAATTACATTCCCAACCGCGTGGCACAACAACTGGCGGGCAAACAGTCGTTGCTGATTGGCGTTGCCACCTCCAGTCTGGCCC
1820
CAAAGACGCTTTTGCGCCCTTTTTCACCTTCGCCGCTACCGCCTCGACTTAATGTAAGGGTTGGCGCACCGTGTTGTTGACCGCCCGTTTGTCAGCAACGACTAACCGCAACGGTGGAGGTCAGACCGGG
lac I
M A E L N Y I P N R V A Q Q L A G K Q S L L I G V A T S S L A
TGCACGCGCCGTCGCAAATTGTCGCGGCGATTAAATCTCGCGCCGATCAACTGGGTGCCAGCGTGGTGGTGTCGATGGTAGAACGAAGCGGCGTCGAAGCCTGTAAAGCGGCGGTGCACAATCTTCTCGC
1950
ACGTGCGCGCGCAGCGTTTAACAGCGCCGCTAATTTAGAGCGCGGCTAGTTGACCCACGGTCGCACCACCACAGCTACCATCTTGCTTCGCCGCAGCTTCGGACATTTCGCCGCCACGTGTTAGAAGAGCG
lac I
L H A P S Q I V A A I K S R A D Q L G A S V V V S M V E R S G V E A C K A A V H N L L A
GCAACGCGTCAGTGGGCTGATCATTAACTATCCGCTGGATGACCAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGTTCCGGCGTTATTTCTTGATGTCTCTGACCAGACACCCATCAACAGTATT
2080
CGTTGCGCAGTCACCCGACTAGTAATTGATAGGCGACCTACTGGTCCTACGGTAACGACACCTTCGACGGACGTGATTACAAGGCCCCAATAAAGAACTACAGAGACTGGTCTGTGGGTAGTTGTCATAA
lac I
Q R V S G L I I N Y P L D D Q D A I A V E A A C T N V P A L F L D V S D Q T P I N S I
ATTTTCTCCCATGAAGACGGTACGCGACTGGGCGTGGAGCATCTGGTCGCATTGGGTCACCAGCAAATCGCGCTGTTAGCGGGCCCATTAAGTTCTGTCTCGGCGCGTCTGCGTCTGGCTGGCTGGCATA
2210
TAAAAGAGGGTACTTCTGCCATGCGCTGACCCGCACCTCGTAGACCAGCGTAACCCAGTGGTCGTTTAGCGCGACAATCGCCCCGGGTAATTCAAGACAGAGCCGCGCAGACGCAGACCGACCGACCGTAT
lac I
I F S H E D G T R L G V E H L V A L G H Q Q I A L L A G P L S S V S A R L R L A G W H

FIG.67C

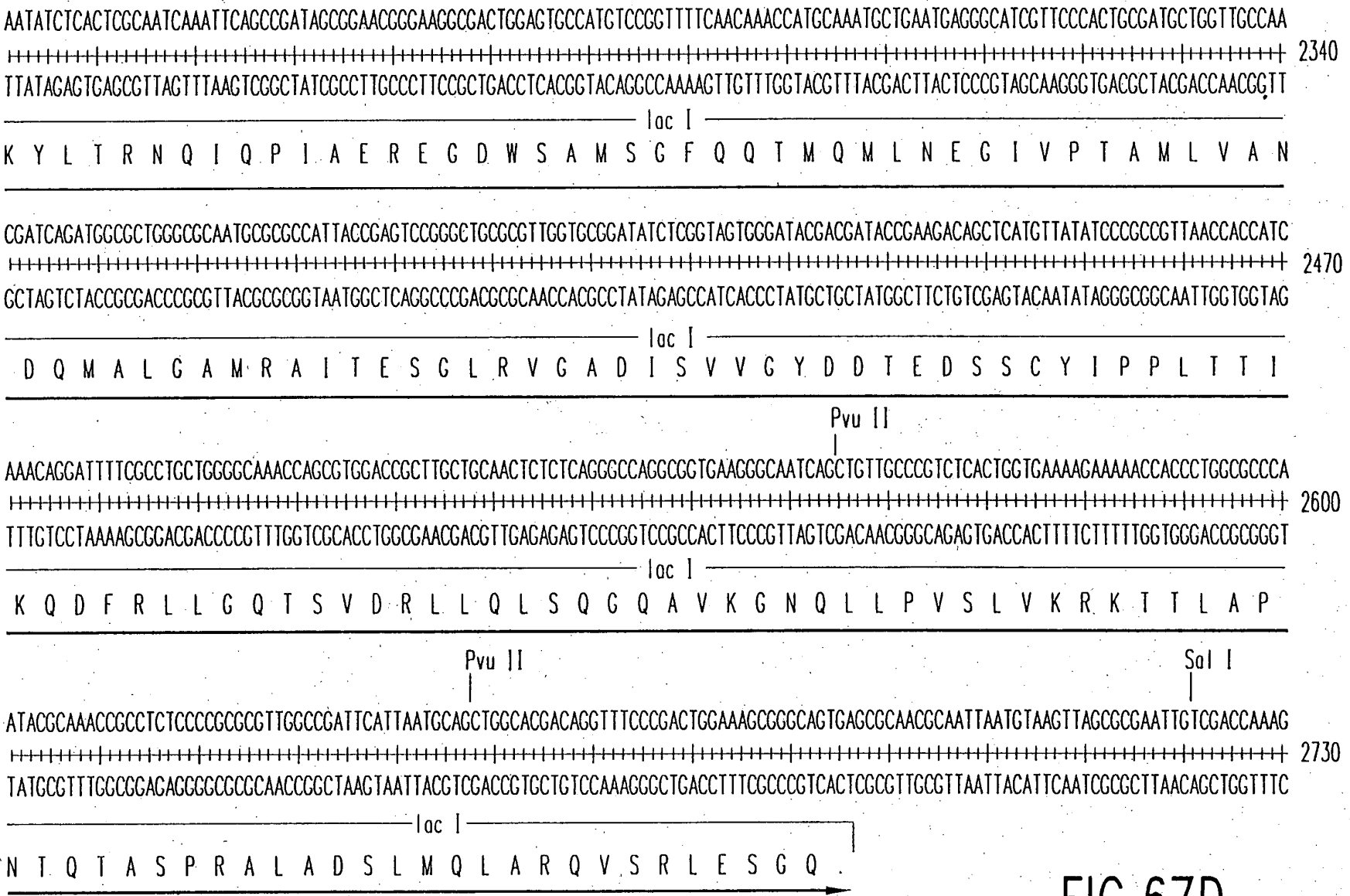

AATATCTCACTCGCAATCAAATTCAGCCGATAGCGGAACGGGAAGGCGACTGGAGTGCCATGTCCGGTTTTCAACAAACCATGCAAATGCTGAATGAGGGCATCGTTCCCACTGCGATGCTGGTTGCCAA
TTATAGAGTGAGCGTTAGTTTAAGTCGGCTATCGCCTTGCCCTTCCGCTGACCTCACGGTACAGGCCAAAAGTTGTTTGGTACGTTTACGACTTACTCCCGTAGCAAGGGTGACGCTACGACCAACGGTT
2340
lac I
K Y L T R N Q I Q P I A E R E G D W S A M S G F Q Q T M Q M L N E G I V P T A M L V A N
CGATCAGATGGCGCTGGGCGCAATGCGCGCCATTACCGAGTCCGGGCTGCGCGTTGGTGCGGATATCTCGGTAGTGGGATACGACGATACCGAAGACAGCTCATGTTATATCCCGCCGTTAACCACCATC
GCTAGTCTACCGCGACCCGCGTTACGCGCGGTAATGGCTCAGGCCCGACGCGCAACCACGCCTATAGAGCCATCACCCTATGCTGCTATGGCTTCTGTCGAGTACAATATAGGGCGGCAATTGGTGGTAG
2470
lac I
D Q M A L G A M R A I T E S G L R V G A D I S V V G Y D D T E D S S C Y I P P L T T I
Pvu II
AAACAGGATTTTCGCCTGCTGGGGCAAACCAGCGTGGACCGCTTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCA
TTTGTCCTAAAAGCGGACGACCCCGTTTGGTCGCACCTGGCGAACGACGTTGAGAGAGTCCCGGTCCGCCACTTCCCGTTAGTCGACAACGGGCAGAGTGACCACTTTTCTTTTTGGTGGGACCGCGGGT
2600
lac I
K Q D F R L L G Q T S V D R L L Q L S Q G Q A V K G N Q L L P V S L V K R K T T L A P
Pvu II
Sal I
ATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTAAGTTAGCGCGAATTGTCGACCAAAG
TATGCGTTTGGCGGAGAGGGCGCGCCAACCGGCTAAGTAATTACGTCGACCGTGCTGTCCAAAGGCCTGACCTTTCGCCCGTCACTCGCGTTGCGTTAATTACATTCAATCGCGCTTAACAGCTGGTTTC
2730
lac I
N T Q T A S P R A L A D S L M Q L A R Q V S R L E S G Q .

FIG.67D

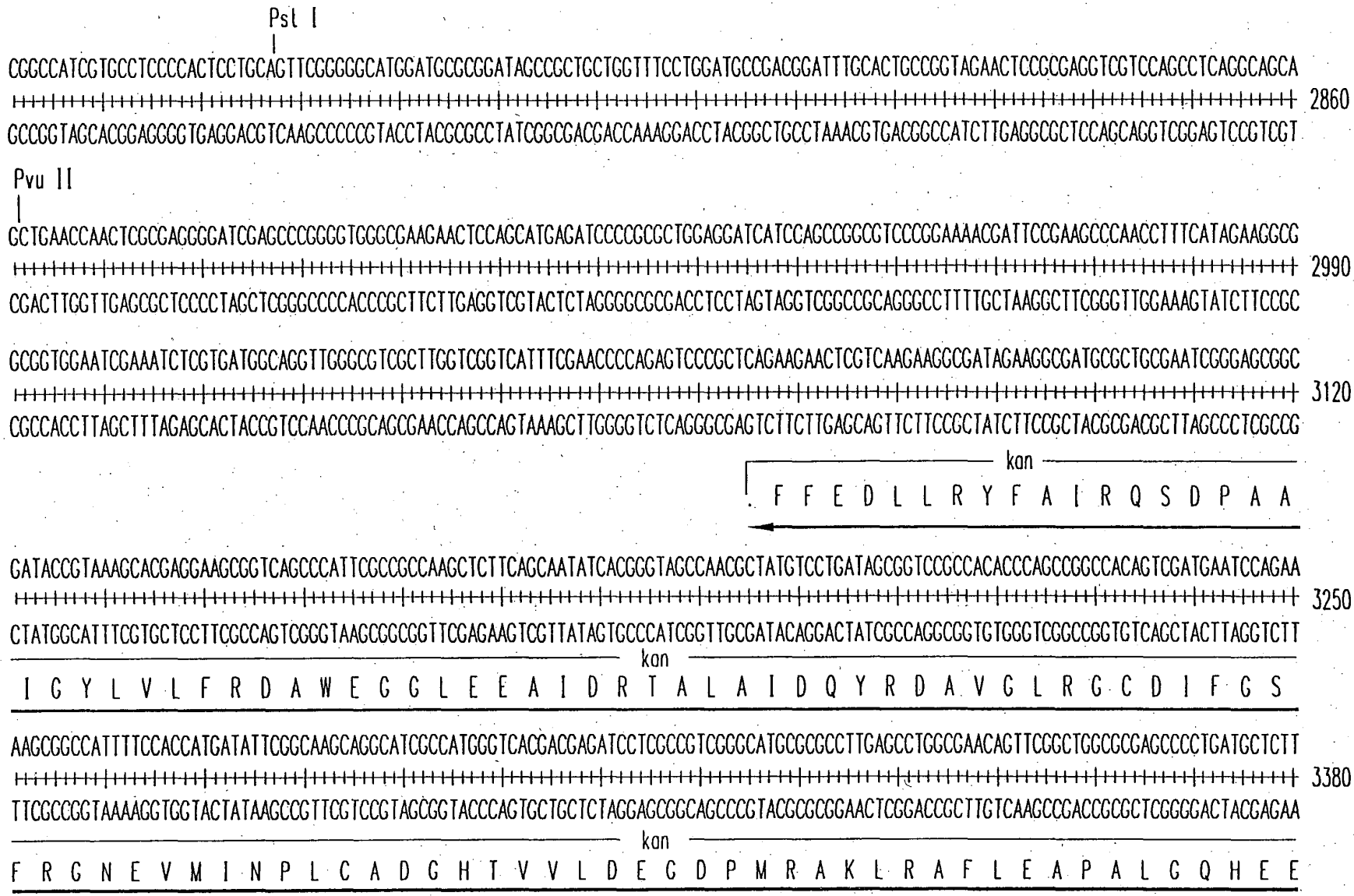

Pst I
CGGCCATCGTGCCTCCCCACTCCTGCAGTTCGGGGGCATGGATGCGCGGATAGCCGCTGCTGGTTTCCTGGATGCCGACGGATTTGCACTGCCGGTAGAACTCCGCGAGGTCGTCCAGCCTCAGGCAGCA
2860
GCCGGTAGCACGGAGGGCTGAGGACGTCAAGCCCCCGTACCTACGCGCCTATCGGCGACGACCAAAGGACCTACGGCTGCCTAAACGTGACGGCCATCTTGAGGCGCTCCAGCAGGTCGGAGTCCGTCGT
Pvu II
GCTGAACCAACTCGCGAGGGATCGAGCCCGGGGTGGCGCAAGAACTCCAGCATGAGATCCCCGCGCTGGAGGATCATCCAGCCGGCGTCCCGGAAAACGATTCCGAAGCCCAACCTTTCATAGAAGGCG
2990
CGACTTGGTTGAGCGCTCCCCTAGCTCGGGCCCCACCCGCTTCTTGAGGTCGTACTCTAGGGCGCCGACCTCCTAGTAGGTCGGCCGCAGGGCCTTTTGCTAAGGCTTCGGGTTGGAAAGTATCTTCCGC
GCGGTGGAATCGAAATCTCGTGATGGCAGGTTGGGCGTCGCTTGGTCGGTCATTTCGAACCCCAGAGTCCCGCTCAGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATGCGCTGCGAATCGGGAGCGGC
3120
CGCCACCTTAGCTTTAGAGCACTACCGTCCAACCCGCAGCGAACCAGCCAGTAAAGCTTGGGGTCTCAGGGCGAGTCTTCTTGAGCAGTTCTTCCGCTATCTTCCGCTACGCGACGCTTAGCCCTCGCCG
kan
. F F E D L L R Y F A I R Q S D P A A
GATACCGTAAAGCACGAGGAAGCGGTCAGCCCATTCGCCGCCAAGCTCTTCAGCAATATCACGGGTAGCCAACGCTATGTCCTGATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATGAATCCAGAA
3250
CTATGGCATTTCGTGCTCCTTCGCCAGTCGGGTAAGCGGCGGTTCGAGAAGTCGTTATAGTGCCCATCGGTTGCGATACAGGACTATCGCCAGGCGGTGTGGGTCGGCCGGTGTCAGCTACTTAGGTCTT
kan
I G Y L V L F R D A W E G G L E E A I D R T A L A I D Q Y R D A V G L R G C D I F G S
AAGCGGCCATTTTCCACCATGATATTCGGCAAGCAGGCATCGCCATGGGTCACGACGAGATCCTCGCCGTCGGGCATGCGCGCCTTGAGCCTGGCGAACAGTTCGGCTGGCGCGAGCCCCTGATGCTCTT
3380
TTCGCCGGTAAAAGGTGGTACTATAAGCCGTTCGTCCGTAGCGGTACCCAGTGCTGCTCTAGGAGCGGCAGCCCGTACGCGCGGAACTCGGACCGCTTGTCAAGCCGACCGCGCTCGGGGACTACGAGAA
kan
F R G N E V M I N P L C A D G H T V V L D E G D P M R A K L R A F L E A P A L G Q H E E

FIG.67E

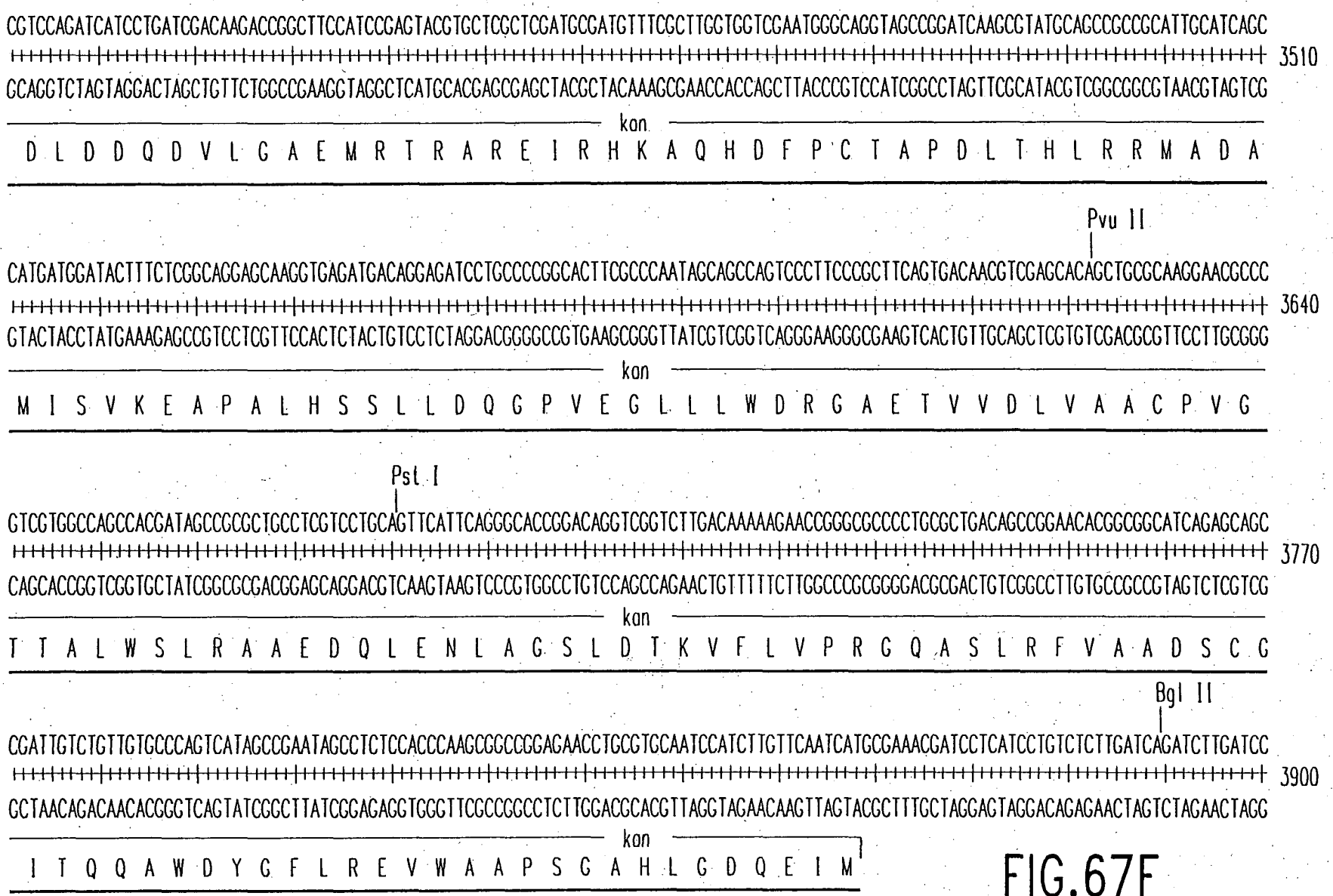

CGTCCAGATCATCCTGATCGACAAGACCGGCTTCCATCCGAGTACGTGCTCGCTCGATGCGATGTTTCGCTTGGTGGTCGAATGGGCAGGTAGCCGGATCAAGCGTATGCAGCCGCCGCATTGCATCAGC
3510
GCAGGTCTAGTAGGACTAGCTGTTCTGGCCGAAGGTAGGCTCATGCACGAGCGAGCTACGCTACAAAGCGAACCACCAGCTTACCCGTCCATCGGCCTAGTTCGCATACGTCGGCGGCGTAACGTAGTCG
kan
D L D D Q D V L G A E M R T R A R E I R H K A Q H D F P C T A P D L T H L R R M A D A
Pvu II
CATGATGGATACTTTCTCGGCAGGAGCAAGGTGAGATGACAGGAGATCCTGCCCCGGCACTTCGCCCAATAGCAGCCAGTCCCTTCCCGCTTCAGTGACAACGTCGAGCACAGCTGCGCAAGGAACGCCC
3640
GTACTACCTATGAAAGAGCCGTCCTCGTTCCACTCTACTGTCCTCTAGGACGGGGCCGTGAAGCGGGTTATCGTCGGTCAGGGAAGGGCGAAGTCACTGTTGCAGCTCGTGTCGACGCGTTCCTTGCGGG
kan
M I S V K E A P A L H S S L L D Q G P V E G L L L W D R G A E T V V D L V A A C P V G
Pst I
GTCGTGGCCAGCCACGATAGCCGCGCTGCCTCGTCCTGCAGTTCATTCAGGGCACCGGACAGGTCGGTCTTGACAAAAAGAACCGGGCGCCCCTGCGCTGACAGCCGGAACACGGCGGCATCAGAGCAGC
3770
CAGCACCGGTCGGTGCTATCGGCGCGACGGAGCAGGACGTCAAGTAAGTCCCGTGGCCTGTCCAGCCAGAACTGTTTTTCTTGGCCCGCGGGGACGCGACTGTCGGCCTTGTGCCGCCGTAGTCTCGTCG
kan
T T A L W S L R A A E D Q L E N L A G S L D T K V F L V P R G Q A S L R F V A A D S C G
Bgl II
CGATTGTCTGTTGTGCCCAGTCATAGCCGAATAGCCTCTCCACCCAAGCGGCCGGAGAACCTGCGTGCAATCCATCTTGTTCAATCATGCGAAACGATCCTCATCCTGTCTCTTGATCAGATCTTGATCC
3900
GCTAACAGACAACACGGGTCAGTATCGGCTTATCGGAGAGGTGGGTTCGCCGGCCTCTTGGACGCACGTTAGGTAGAACAAGTTAGTACGCTTTGCTAGGAGTAGGACAGAGAACTAGTCTAGAACTAGG
kan
I T Q Q A W D Y G F L R E V W A A P S G A H L G D Q E I M

FIG.67F

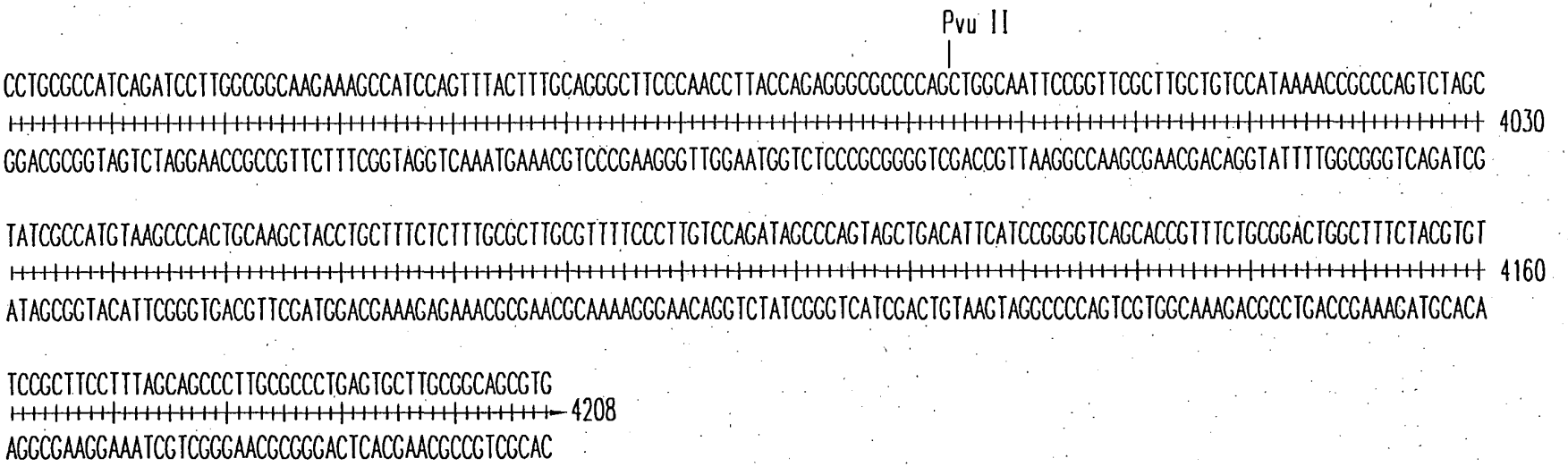

Pvu II
CCTGCGCCATCAGATCCTTGGCGGCAAGAAAGCCATCCAGTTTACTTTGCAGGGCTTCCCAACCTTACCAGAGGGCGCCCCAGCTGGCAATTCCGGTTCGCTTGCTGTCCATAAAACCGCCCAGTCTAGC
4030
GGACGCGGTAGTCTAGGAACCGCCGTTCTTTCGGTAGGTCAAATGAAACGTCCCGAAGGGTTGGAATGGTCTCCCGCGGGGTCGACCGTTAAGGCCAAGCGAACGACAGGTATTTTGGCGGGTCAGATCG
TATCGCCATGTAAGCCCACTGCAAGCTACCTGCTTTCTCTTTGCGCTTGCGTTTTCCCTTGTCCAGATAGCCCAGTAGCTGACATTCATCCGGGGTCAGCACCGTTTCTGCGGACTGGCTTTCTACGTGT
4160
ATAGCGGTACATTCCGGTGACGTTCCATGGACGAAAGAGAAACGCGAACGCAAAAGGGAACAGGTCTATCGGGTCATCGACTGTAAGTAGGCCCCAGTCGTGGCAAAGACGCCTGACCGAAAGATGCACA
TCCGCTTCCTTTAGCAGCCCTTGCGCCCTGAGTGCTTGCGGCAGCGTG
4208
AGGCGAAGGAAATCGTCGGGAACGCGGGACTCACGAACGCCGTCGCAC

FIG.67G